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13. ABSTRACT ***(Maximum 200 Words)***

There is tremendous variability in the amount of hearing loss individuals develop from a given noise exposure. The reasons for individual differences in susceptibility to noise-induced hearing loss (NIHL) are largely unknown, but may include factors such as levels of endogenous antioxidant enzymes or steroid hormones. Our experiments are focusing on the effects of steroid hormones on susceptibility to NIHL in chinchillas. Chinchillas treated with 17-β-estradiol prior to noise exposure developed less permanent hearing loss than controls. The protective effects of estradiol were found in two separate experiments, one utilizing a continuous noise (octave band noise with a center frequency of 4 kHz, at 105 dB SPL for 4 hours), and the other an impulse noise simulating M16 rifle fire. These experiments support the hypothesis that endogenous levels of estrogen could influence individual susceptibility to NIHL.

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Sandra L. McFadden 9-28-99

PI - Signature Date

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1. INTRODUCTION

All tasks of Technical Objective #1 in the approved Statement of Work were completed during the first two years of the project. In the third and final year of the project, significant progress was made on experiments outlined under Technical Objective #2. A one-year no-cost extension of the grant has been received, so that all tasks of Technical Objective #2 can be completed as outlined in the Statement of Work. The experiments currently being conducted are designed to explore the effects of steroid hormones on noise-induced threshold shifts and hair cell losses in chinchillas. This report describes the advances made toward fulfilling the tasks of Technical Objective #2 for the funding period from Sept. 23,1998 to Sept. 22, 1999. Specifically, this report describes experiments in which chinchillas were given estrogen (17-β-estradiol) prior to noise exposure. The results show that estrogen treatment reduces threshold shifts associated with exposure to two different types of noise, impulse noise simulating M16 rifle fire and continuous noise. The findings from the chinchilla offer insights into factors that may contribute to individual differences in noise susceptibility in humans.

2. BODY

Summary of task objectives for Technical Objective #2: Test auditory sensitivity before and after noise exposure in animals treated with steroid hormones, and examine the effects of steroid hormones on noise-induced hair cell loss.

2.1. METHODS

All procedures were reviewed and approved by the University of Buffalo Animal Care and Use Committee, and conformed to NIH guidelines for the humane treatment of laboratory animals.

2.1.1. Subjects and surgery

Subjects were 37 chinchillas (*Chinchilla langier*) between 1 and 3 years of age. Animals were deeply anesthetized with an intramuscular injection of ketamine hydrochloride (Ketaset; 56 mg/kg) and acepromazine (Promace; 0.56 mg/kg). Tungsten electrodes were implanted into the right and/or left inferior colliculus (IC) and the rostral cranium for recording auditory evoked potentials (EVPs). Details of the surgical procedures are provided in Appendix I (McFadden et al., 1999). Following surgery, the animals recovered in a quiet animal colony for at least one week prior to testing.

2.1.2. EVP testing

The auditory sensitivity of each animal was determined from EVP thresholds. All testing was conducted in a sound attenuating booth (Industrial Acoustics Corp. 400) lined with sound absorbing foam panels. The awake chinchilla was placed in a custom-designed tube (Snyder and Salvi, 1994) that held its head at a constant orientation within the calibrated sound field. Stimuli consisted of 10 ms tones (2 ms cosine rise/fall ramp, alternating phase) at octave intervals from 0.5 to 16 kHz, presented at a rate of 19/sec. Stimulus level was incremented in 5 dB steps from below threshold to 80 dB SPL. Details of the stimuli and recording procedures can be found in Appendix I (McFadden et al., 1999).

2.1.3. Noise stimuli and acoustic calibration

Animals were exposed to either impulse noise or octave band noise (OBN) with a center frequency of 4 kHz. The impulse noise simulated impulses created by a U.S. Army M16A1 rifle (Danielson, Henderson, Gratton, Bianchi, and Salvi, 1991). The impulses were generated digitally, attenuated (HP 350D), amplified (NAD 2200), and delivered to a compression driver (JBL 2446) coupled to a sound delivery tube (5 cm dia X 20 cm) whose end was cut at a 45° angle to broaden the range of resonance (Danielson et al., 1991). An animal in a restraint tube was placed 10 cm away from the acoustic driver and exposed to 50 pairs of impulses (100 total). Impulses in each pair were spaced 50 ms apart, and there was a 1000 ms interval between the onset of each pair (Henselman et al., 1994). The duration of the exposure was 50 sec. For calibration of the impulse noise, a 1/8" microphone (Bruel and Kjaer Model 4138) was placed at the position that would be occupied by a restrained animal. The voltage corresponding to a 114 dB, 250 Hz tone produced by a pistonphone coupled to the microphone was determined, and used to calculate the desired voltage for a 150 dB peak SPL signal. The attenuation was adjusted to produce the desired voltage.

The 4 kHz OBN was generated digitally, attenuated (HP 350D), amplified (NAD 2200), and delivered to a compression driver (JBL 2446) suspended from the ceiling of the sound booth. During exposure, animals were placed in individual cages beneath the loudspeaker and provided free access to food and water. The exposure level, calibrated with a Type I sound level meter and condenser microphone, was 105 dB SPL. Exposure duration was 4 hr.

2.1.4. Experimental protocol

Animals were randomly assigned to estradiol treatment groups, a vehicle control group, or an untreated control group. Animals in estradiol treatment groups received daily subcutaneous injections of 17-β-estradiol (Sigma Chemicals) dissolved in olive oil vehicle for 7-14 consecutive days. Animals in the vehicle control group received an equal volume of vehicle alone on the same schedule as estradiol-treated animals. IC-EVP thresholds were measured twice before treatment, and at various times relative to treatment and noise exposure. Post-noise measurements were made at 15 min, 24 hr, 7 days, and 14-21 days.

After all measurements had been obtained, chinchillas were deeply anesthetized with sodium pentobarbital (Somlethal, 100 mg/kg i.p.) and decapitated. The cochleas were quickly removed, stained with a succinate dehydrogenase (SDH) staining solution, and post-fixed with 10% formalin (see details in Appendix I, McFadden et al., 1999). Cochleas were dissected from the apex to the base, mounted in sections in glycerin on microscope slides, coverslipped, and examined using light microscopy (400X magnification). The numbers of missing OHCs and IHCs were determined for successive segments of the organ of Corti. Individual cochleograms were constructed to show the percentage of hair cells missing as a function of distance from the apex of the cochlea. Percent hair cells missing was referenced to our lab standards based on average hair cell counts from 9 cochleas of young (<1 yr old), healthy chinchillas. Percent distance from the apex was converted to frequency using the frequency-place map of Greenwood (1990).

2.1.5. Data analyses

Data analyses were geared toward answering two questions. First, does estradiol treatment affect basic auditory sensitivity? Second, does estradiol influence susceptibility to impulse noise or continuous noise? Analyses of variance (ANOVAs) and Student t-tests were used to assess differences between means. The dependent variables were IC-EVP thresholds and threshold shifts at various times after treatment or noise exposure. All statistical tests were evaluated using a 0.05 criterion of significance.

2.1.6. Estradiol assays

Blood samples were collected from deeply anesthetized chinchillas prior to cochlear histology. The blood samples were centrifuged to separate serum for estradiol assays. Samples were treated with a steroid displacement reagent to free estradiol bound to transport proteins in the serum. Estradiol levels were measured using an Enzyme Immunoassay kit (Assay Designs Inc., product number 90108). Microplates from the assay were read using a microplate reader from Bio-Rad Laboratories, Inc. (Model 3550-UV). All samples were run in triplicate to ensure reliability.

2.2. RESULTS AND DISCUSSION

2.2.1. Preliminary results of estradiol assays

The following table presents measured levels of estradiol in picograms/ml serum. For each subject listed, the value is the average of 3 assays.

Females

109.01
256.58
258.36
263.92
312.76

M=240.12; sd= 76.87

Males

40.82
66.92
69.98
82.40

M=65.03; sd= 17.47

Two aspects of these results are particularly interesting. First, the levels for female and male chinchillas are in the range reported for humans. This supports the use of the chinchilla in studies of hormone effects on hearing. Second, females have higher levels and are much more variable than males. Because individuals show a wide range of variability, it will be possible in

future studies to correlate endogenous levels of hormones with susceptibility to NIHL in both treated and untreated populations.

2.2.2. Experiments with estradiol pre-treatment

The effects of 17-β-estradiol on susceptibility to NIHL were studied in two experiments. In Experiment I, chinchillas in the estradiol groups were given daily injections of estradiol in olive oil for 1-2 weeks before exposure, for total doses of 200-265 mg. Animals in a vehicle control group received injections of the olive oil vehicle alone. Thresholds measured on Days 2, 4 and 7during the course of hormone treatment were not different from those measured before treatment, indicating that short-term estradiol treatment has no direct effect on auditory sensitivity. All animals were exposed to impulse noise, and EVPs were measured 15 min, 24 hr, 7 days and 14 days after exposure.

Permanent threshold shifts are shown in Figure 1. The animals in the vehicle control group had PTS ranging from 20 dB at 0.5 kHz to 55 dB at 8 kHz. Chinchillas pre-treated with estradiol had significantly less PTS at frequencies between 2 and 8 kHz. On average, chinchillas pre-treated with estradiol had PTS values ranging from 15 dB at 0.5 kHz to 30 dB at 2 kHz. At 8 kHz, estradiol-treated chinchillas had less than 20 dB hearing loss—a "savings" of 35 dB relative

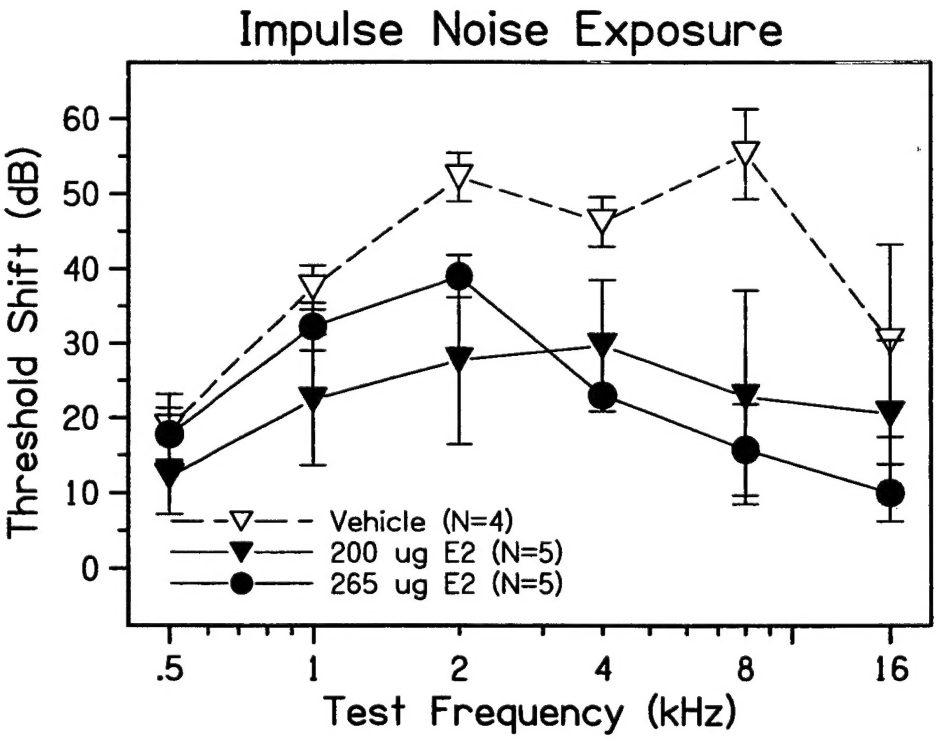

Figure 1. Permanent threshold shifts of animals exposed to M16 rifle fire.

to controls. These results indicate that estradiol treatment provides protection from threshold shifts caused by impulse noise.

Hair cell losses are shown in Figure 2. Inner hair cell losses are shown in the top panel; outer hair cell losses are compared in the bottom panel. Overall, there was no dramatic difference in hair cell loss between estradiol-treated animals and the vehicle control animals. Although

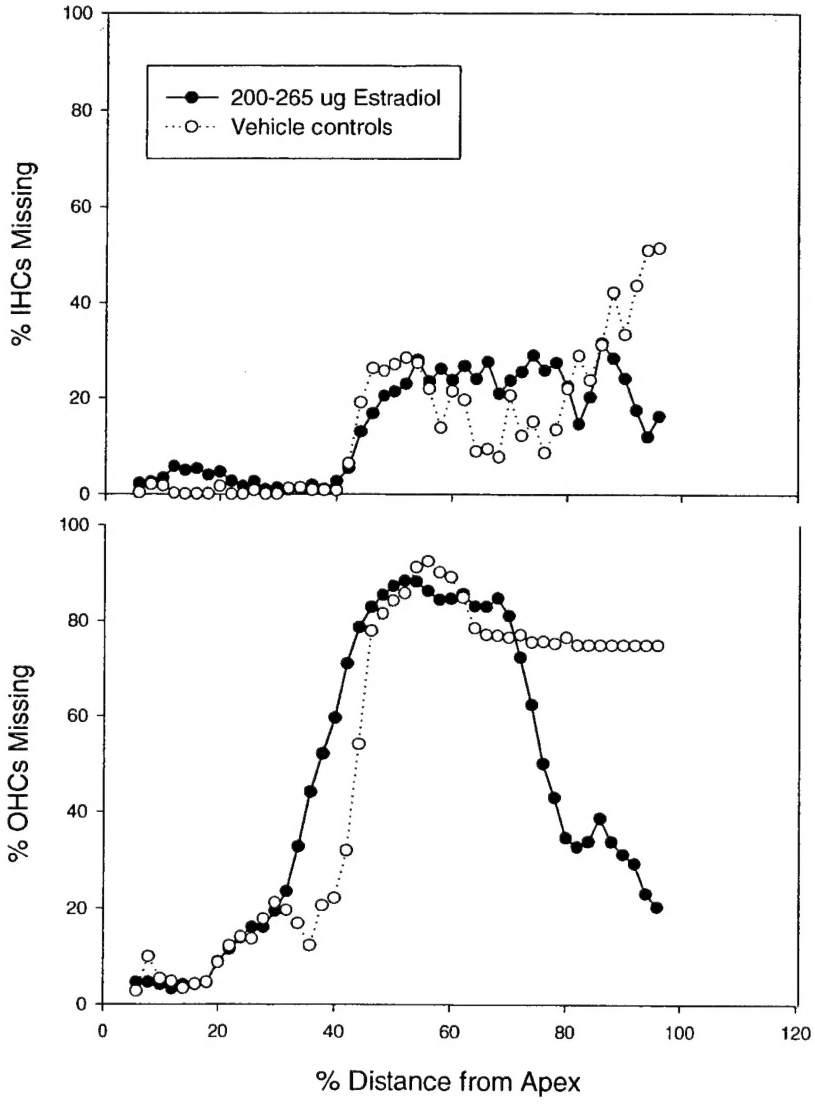

Figure 2. Hair cell losses following impulse noise exposure. Upper panel shows inner hair cell loss; lower panel shows outer hair cell loss.

estradiol-treated animals had much less hair cell loss in the basal-most 10-20% of the cochlea, differences in other regions were minor. This finding is interesting because it suggests that estradiol may act on the stria vascularis rather than the organ of Corti. However, it should be noted that it is not unusual to see a lack of correspondence between hair cell loss and hearing loss

(Hamernik et al., 1989; Boettcher et al., 1992; McFadden et al., 1997), and the meaning of this dissociation is not clear.

In Experiment II, chinchillas in the estradiol groups received estradiol for 7 days prior to exposure. Total estradiol dose was either 100 μg (N=5) or 725 μg (N=6). Animals in a control group received no treatment prior to noise. All animals were exposed to 4 kHz octave band noise at a level of 105 dB SPL for 4 hours. This is the same exposure that was used in a recent study of protection using the chemical R-phenylisopropyladenosine (R-PIA) (Hu et al., 1997). In that study, R-PIA was placed on the round window of the cochlea and wicked off after 30 minutes. Chinchillas treated with R-PIA developed significantly less PTS than controls that had received a drop of saline on the round window. The results from the Hu et al. experiment are compared to the current results to provide a perspective on the magnitude of protection afforded by systemic treatment with estradiol.

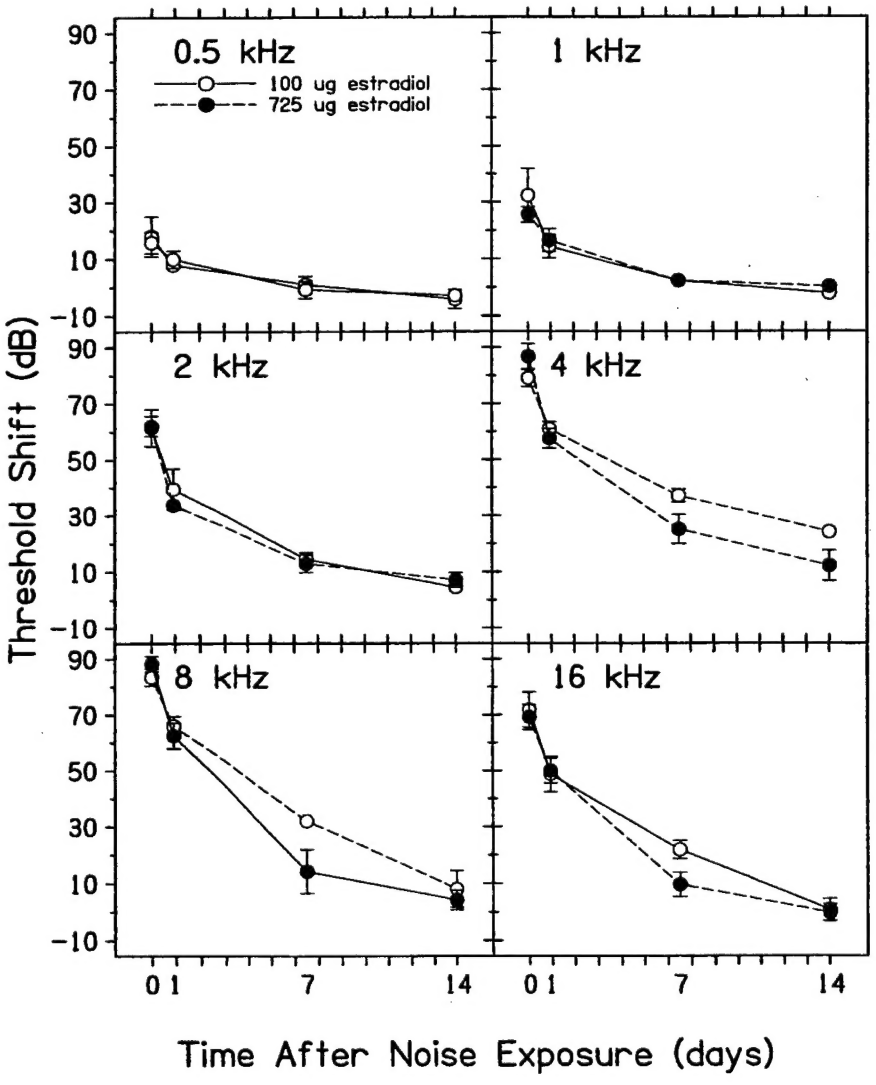

Figure 3. Recovery of sensitivity after exposure to 4 kHz octave band noise.

Figure 3 shows recovery of hearing over time for chinchillas treated with estradiol. Threshold shifts at 14 days represent PTS. The recovery curves are very similar for animals in the low dose

and the high dose groups. However, animals in the high dose group had less threshold shift at high frequencies than animals in the low dose group. The greatest divergence between treatment groups is seen at 4 and 8 kHz on Day 7. Differences at 4 kHz persist at Day 14. Figure 4 shows PTS as a function of frequency for treated animals and controls. Results from Hu et al. (1997) are shown for comparison. It is clear that estradiol treatment reduced PTS relative to controls. The high dose of estradiol produced savings equivalent to those seen following R-PIA treatment in the Hu et al. (1997) experiment. This is important, because the protective effects achieved with R-PIA involved invasive surgery, whereas the protective effects of estradiol were achieved with simple systemic treatment.

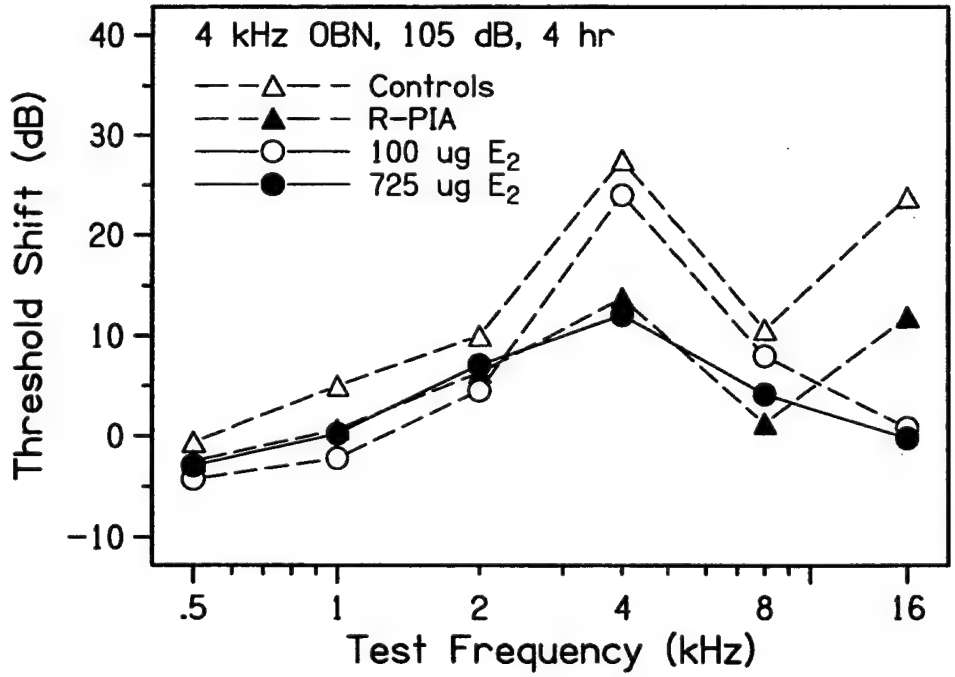

Figure 4. Permanent threshold shifts caused by 4 kHz octave band noise.

Hair cell losses are shown in Figure 5. As with the previous experiment (Fig. 2), there were no remarkable differences between treated and control groups in inner hair cell loss (upper panel) or outer hair cell loss (lower panel). These results are consistent with the hypothesis that estradiol acts on vascular tissue of the cochlea rather than on the hair cells themselves.

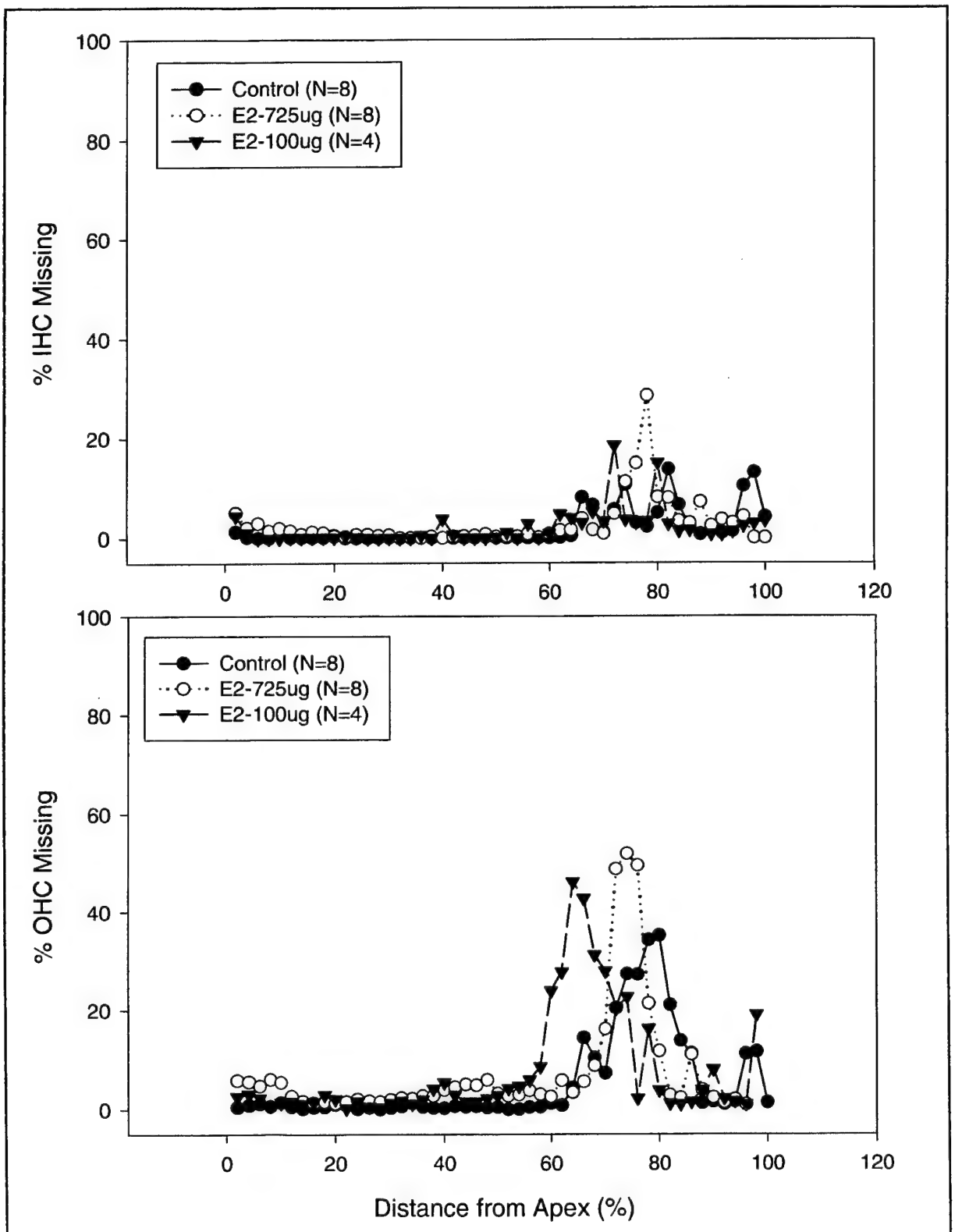

Figure 5. Hair cell losses after exposure to 4 kHz octave band noise.

Like R-PIA, steroid hormones can act through many different routes, each of which could be important for modulating susceptibility to NIHL. Estradiol can potentiate the activity of gamma-aminobutyric acid, a ubiquitous inhibitory neurotransmitter of the central nervous system; it can affect neuronal activity via changes in cellular neurochemistry and morphology; it can act on cell membranes to alter permeability to neurotransmitters, precursors and receptor functioning; it can act directly as an antioxidant; and it can influence the bioactivity of other antioxidants and blood flow promoters such as nitric oxide (Arnal et al., 1996; Ayres et al., 1996; Behl et al., 1995; Chadwick and Widdows, 1990; Goodman et al., 1996; Romer et al., 1997; Ruiz-Larrea et al., 1994).

2.2.3. PROBLEMS

Progress toward completing all experiments outlined for the third year of the grant was impeded by several unforeseeable problems. Most of these revolved around animal availability and health. The first animal problem arose when our regular chinchilla supplier ran out of chinchillas early in the year, forcing us to locate a different supplier. The first batch of animals we purchased from the new breeder was of excellent quality, but subsequent batches have been of very mixed quality, and a large number of animals have been unsuitable for our experiments. Our regular supplier now has a minimum order size of 30. This limits how frequently we can order animals, due to our limited cage space. We have also had problems getting an adequate number of female chinchillas for our experiments.

Progress was also slowed down by changes in personnel and the need to develop new techniques for assaying hormone levels from serum samples. During the course of the year, two new surgeons were trained to perform the electrode implantation surgeries, a new technician was trained for histological evaluations of cochleas, and two technicians were trained in animal handling and testing procedures.

Due to the problems described above, we applied for and received a one-year no-cost extension on the grant. This additional year will allow us to finish all experiments as originally outlined in our Statement of Work.

3. KEY RESEARCH ACCOMPLISHMENTS—Year 3

* Estradiol levels were determined from serum samples of female and male chinchillas, and found to vary on a continuum comparable to humans.

* The protective effects of estradiol on noise-induced hearing loss were demonstrated in two separate experiments that utilized different noise exposure conditions. Estradiol reduced NIHL from octave band noise centered at 4 kHz and impulse noise simulating M16 rifle fire.

* Estradiol was determined to have protective effects similar to another known otoprotectant, R-PIA. Unlike R-PIA, however, the protective effects of estradiol were achieved with simple systemic treatment rather than invasive surgery.

* The mechanism of estradiol protection does not appear to involve sparing of cochlear hair cells. Estradiol may exert its effects on the stria vascularis, or by acting as an antioxidant or a modulator of cochlear neurotransmitter substances.

4. REPORTABLE OUTCOMES: Manuscripts, abstracts & presentations

1. McFadden, S.L., Henselman, L.W., and Zheng, X.Y. (1999). Sex differences in auditory sensitivity of chinchillas before and after exposure to impulse noise. ***Ear Hear.* 20**, 164-174.

2. McFadden, S.L., Zheng, X.Y., and Ding, D.L. Conditioning-induced protection from impulse noise in female and male chinchillas. ***J. Acoust. Soc. Am.*** (submitted 7/26/99).

3. McFadden, S.L., Zheng, X.Y., and Ding, D.L. Sex differences in threshold shifts and hair cell loss in chinchillas exposed to simulated military noises. (manuscript in preparation; to be submitted to ***Noise and Health***)

4. McFadden, S.L. Overview of research on steroid hormones and noise-induced hearing loss. ***Lake Ontario Hearing Meeting***, Syracuse University, June 15, 1999.

5. McFadden, S.L., Zheng, X.Y., Ding, D.L., and Henderson, D. (1999) Differences between female and male chinchillas in susceptibility to noise-induced hearing loss. ***Assoc. Res. Otolaryngol. Abstr.* 610**.

6. Lockwood, D., McFadden, S.L., Jiang, H., and Rosenberg, L. Systemic treatment with estradiol reduces noise-induced hearing loss in the chinchilla. ***Assoc. Res. Otolaryngol. Abstr.*** (submitted 10/1/99).

7. McFadden, S.L. and Henderson, D. (1999) Recent advances in understanding and preventing noise-induced hearing loss. ***Current Opin. Otolaryngol. Head Neck Surg.***, 7(5).

5. CONCLUSIONS

The results from the experiments described here have important practical and theoretical implications. From a theoretical standpoint, they provide a much-needed perspective on the role of steroid hormones on normal physiology and function, thereby increasing our understanding of basic auditory system physiology. Gender differences have frequently been reported in humans, but the basis for these differences has remained elusive. Often, sex differences have been attributed to differences in noise exposure history, as males are more often engaged in occupational and recreational activities that involve high-level noise. However, our findings argue for more fundamental, inherent differences related to biochemical and hormonal factors. Based on our findings, it is reasonable to hypothesize that individuals with high estrogen levels will be less susceptible to NIHL than individuals with low estrogen levels.

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LIST OF APPENDICES

I. Paper published in *Ear and Hearing.*

II. Abstract submitted to Association for Research in Otolaryngology, 2000.

III. Poster presented at Association for Research in Otolaryngology, 1999.

IV. Manuscript submitted to *Journal of the Acoustical Society of America.*

Sex Differences in Auditory Sensitivity of Chinchillas Before and After Exposure to Impulse Noise

Sandra L. McFadden, Lynn W. Henselman, and Xiang-Y. Zheng

Objective: **To determine if chinchillas exhibit sex differences in 1) basic auditory sensitivity and 2) susceptibility to cochlear damage and hearing loss from high-level impulse noise.**

Design: **The auditory sensitivity of 73 chinchillas was assessed by measuring evoked potentials from electrodes implanted in the inferior colliculus (IC-EVPs) and cubic ($2f_1$-f_2) distortion product otoacoustic emissions (CDPs). A subgroup of 16 chinchillas were retested after exposure to simulated M16 rifle fire (150 dB pSPL impulse noise). Thresholds and postexposure temporary and permanent threshold shifts were compared as a function of sex and frequency using analysis of variance procedures. Cochleograms, showing the percent of hair cells missing as a function of location on the basilar membrane, were constructed to show inner hair cell (IHC) and outer hair cell (OHC) losses for each group.**

Results: **Female chinchillas had slightly lower high-frequency thresholds, and slightly higher low-frequency thresholds than male chinchillas, but similar IC-EVP and CDP amplitude functions. Significant sex differences were observed after exposure to high-level impulse noise. Overall, female chinchillas developed approximately 10 dB more high-frequency hearing loss, but approximately 5 dB less low-frequency hearing loss than males. Hair cell losses, particularly IHC losses, were substantially less for females as compared with males.**

Conclusions: **The results point to close similarities between chinchillas and humans with regard to sex/gender differences in basic auditory sensitivity before noise exposure, suggesting that the chinchilla may be a good model for exploring the anatomical and physiological bases of these differences. In addition, the results show significant sex differences in the physiological and anatomical response of the chinchilla cochlea to high-level noise. Similar differences in humans could have important implications with regard to military assignments and hearing conservation programs.**

(Ear & Hearing 1999;20;164–174)

Center for Hearing and Deafness (S.L.M., X.Y.Z.), University of Buffalo, Buffalo, New York; and Office of the Inspector General (L.W.H.), Department of Defense, Arlington, Virginia.

Noise-induced hearing loss (NIHL) is a major occupational hazard for military personnel because of the types and levels of noise encountered in training and combat situations (Dancer & Franke, 1986; Henselman, Henderson, Shadoan, Subramaniam, Saunders, & Ohlin, 1995; Henselman, Henderson, Subramaniam, & Sallustio, 1994). Damage to the cochlea can be caused by a variety of acoustic events, ranging from prolonged exposure to continuous noises that cause metabolic and biochemical changes in the cochlea, to relatively brief exposures to high-level impact and impulse noises such as gunfire, cannon fire and explosions, that can produce direct mechanical damage as well (Dancer & Franke, 1986; Henderson, Hamernik, & Sitler, 1974; Henderson, Spongr, Subramaniam, & Campo, 1994). A recognition of the serious consequences of NIHL led the U.S. Air Force to develop the first hearing conservation program (HCP) in 1948. The U.S. Navy and U.S. Army developed similar HCPs in 1955 and 1956, respectively (Henselman et al., 1995). Since their inception, military HCPs have served to increase awareness of the damaging effects of high-level noise exposure. They have also served to reduce the incidence and magnitude of NIHL in military personnel, primarily by mandating the use of personal protection devices such as sound-attenuating ear plugs or earmuffs in high-noise situations. However, NIHL remains a serious problem for military personnel who are exposed to loud noises during training and combat situations in which personal protection devices are either unavailable, impractical or dangerous to use, improperly fitted or worn, or inadequately designed to protect the ear from damage (Dancer, Buck, Parmentier, & Hamery, 1998).

As women become more fully integrated into a variety of military occupational specialties, many will be placed at risk for developing NIHL. It is critical, therefore, that we understand the specific relationship between noise exposure and hearing loss in women, so that appropriate measures for preventing NIHL can be developed and implemented.

Previous studies (Chung, Mason, Gannon, & Willson, 1983; Corso, 1963; Pearson et al., 1995; Ward,

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1966) have reported small differences (generally less than 3 dB) between males and females in auditory sensitivity (i.e., thresholds for detecting pure tones under quiet listening conditions). In general, females tend to have slightly better pure-tone thresholds than males at frequencies above 1 to 2 kHz, whereas males may have slightly better thresholds below 1 to 2 kHz. Small, but consistent gender differences* have also been reported in susceptibility to temporary threshold shifts (TTS) caused by exposure to continuous tones or noise (Axelsson & Lindgren, 1981; Dengerink, Dengerink, Swanson, Thompson, & Chermak, 1984; Petiot & Parrot, 1984; Ward, 1966). In general, experimental studies of TTS in humans have found that males exhibit more TTS than females from low-frequency exposures (below 2 kHz), whereas females exhibit more TTS than males from high-frequency exposures (above 2 kHz). In an early investigation of gender differences in susceptibility to TTS produced by high intensity tones and noise, Ward (1966) conducted 17 experiments with 24 male and 25 female adults. Females showed approximately 30% less TTS than males when the exposure frequency was below 1 kHz, but approximately 30% more TTS when the exposure frequency was above 2 kHz.

The above studies examined TTS rather than the more important issue of permanent threshold shift (PTS) because it is not ethical to intentionally induce PTS in human subjects. Most of what little we know about gender differences in PTS comes from retrospective studies of workers exposed to noise in industrial settings (Berger, Royster, & Thomas, 1978; Gallo & Glorig, 1964). Under these conditions, which typically involve exposure to low-frequency continuous noises, males tend to develop much more hearing loss than females. Both Berger et al. (1964) and Gallo and Glorig (1964) found approximately 20 dB more PTS in males than in females after 9 yr of industrial noise exposure. These results are consistent with the gender differences observed in Ward's (1966) studies of TTS. However, there are no comparable studies of gender differences in PTS produced by exposures to high-level impulse noises that are typically found in military environments. A finding of gender differences in susceptibility to NIHL could have important implications for military assignments and HCPs.

The present study was conducted to determine whether there are systematic differences between female and male chinchillas in 1) basic auditory function, as assessed by inferior colliculus evoked potentials (IC-EVPs) and cubic ($2f_1$-f_2) distortion product otoacoustic emissions (CDPs), and 2) their susceptibility to high-level impulse noise. Basic auditory function was assessed in a relatively large group of chinchillas (N = 73). Susceptibility to impulse noise was examined in a subgroup of these animals (N = 16). Findings from the chinchilla may shed light on gender differences in susceptibility to impact/impulse noise, and offer insights into the anatomical and physiological mechanisms contributing to documented gender differences in humans.

METHODS

All procedures described here were reviewed and approved by the University of Buffalo Animal Care and Use Committee, and conformed to federal guidelines for the humane treatment of laboratory animals.

Subjects

A total of 73 chinchillas (*Chinchilla laniger*; 37 female, 36 male) between 1 and 3 yr of age were used. A subgroup of animals (eight female, eight male) was exposed to impulse noise, and their thresholds were measured at various times after exposure (see below). The chinchilla was used for these studies because it 1) is relatively immune to middle ear infections and diseases that affect hearing (Clark, 1984); 2) has a relatively long life span (12 to 20 yr) with minor age-related cochlear pathology and hearing loss before 8 to 10 yr of age (Bohne, Gruner, & Harding, 1990; McFadden, Campo, Quaranta, & Henderson, 1997); and 3) reacts predictably to anesthesia and tolerates surgery well. Most importantly, the chinchilla has a range of hearing that is more similar to that of humans than most other laboratory animals, particularly in the low frequencies (Heffner & Heffner, 1991; Miller, 1970), which enhances its suitability as a model for studying NIHL (McFadden, Campo, Ding, & Quaranta, 1998). With regard to size, Clark (1984) states that female chinchillas tend to be larger than males. In a small group of our chinchillas (eight female, eight male) for which reliable weights were available, weight differences were minor, but favored females. The average weight of females was 572.2 g (SD = 73.7), versus 563.9 g (SD = 70.0) for males.

Surgical Preparation

Each animal was deeply anesthetized with an intramuscular injection of ketamine hydrochloride (60 mg/kg) and acepromazine (0.5 mg/kg). Chronic recording electrodes were implanted in the left and/or right inferior colliculus (IC), and in the ros-

* The term "gender differences" will be used to refer to male/female differences in humans, and the term "sex differences" will be used to refer to male/female differences in chinchillas and other nonhuman species.

tral cranium (McFadden et al., 1997). Thirteen animals were implanted unilaterally; all others were implanted bilaterally. A small hole was drilled in the dorsal cranium overlying the IC, and a recording electrode mounted on a stereotaxic device was advanced through the IC while the surgeon monitored sound-evoked electrical activity on audio and video monitors. When the electrode had been advanced to a depth that produced clear, large amplitude EVPs, it was cemented to the skull with cyanoacrylic adhesive and dental cement. A short electrode was implanted in the rostral cranium to serve as the common lead for IC-EVP recording. Because the electrodes remain fixed in position, variability associated with changes in electrode placement between tests is eliminated. In addition, the signal to noise ratio is much better with implanted electrodes than with more conventional scalp electrodes, so that thresholds can be determined with greater precision. IC-EVPs recorded from electrodes implanted in this manner yield thresholds that are very close to behavioral thresholds measured in the same animals (Henderson, Hamernik, Salvi, & Ahroon, 1983), and about 15 to 20 dB lower than threshold estimates obtained using subcutaneous electrodes in the same animals (Murphy & Themann, Reference Note 1). After surgery, the animals recovered in a quiet animal colony for at least 1 wk before testing.

Measures of Auditory Function

The auditory sensitivity of each animal was assessed by measuring IC-EVPs. CDPs were also obtained from most animals. All testing was conducted in a sound-attenuating booth (Industrial Acoustics Corp. 400) lined with sound-absorbing foam panels. The awake chinchilla was placed in a custom-designed tube (Snyder & Salvi, 1994) that held its head at a constant orientation within the calibrated sound field.

Stimuli for IC-EVP testing consisted of 10 msec tones (2 msec Blackman rise/fall ramp, alternating phase) at octave intervals from 0.5 to 16 kHz, presented at a rate of 20/sec. Stimuli were generated digitally (93 kHz sampling rate) by a 16 bit D/A converter on a digital signal processing (DSP) board (TMS320C25) in a personal computer and routed through computer-controlled attenuators and impedance matching transformers to a loudspeaker (Realistic 401197) located on the side of the test ear, at a distance of approximately 9 cm from the animal's pinna. The nontest ear was plugged with a foam insert earplug, providing approximately 20 to 40 dB attenuation in addition to the attenuation produced by the animal's head and body obstructing the propagation of sound to the opposite ear. Electrical activity from the IC electrode contralateral to the test ear was amplified (20,000×), filtered (10 to 3000 Hz), and converted to digital signals (50 kHz sampling rate) by an A/D converter on a separate computer DSP board. Stimuli were presented in ascending order of frequency and intensity. Fifty or 100 trials were computer averaged at each stimulus level and the level was incremented in 5 dB steps. Figure 1 illustrates IC-EVP waveforms (raw data) obtained from a normal chinchilla.

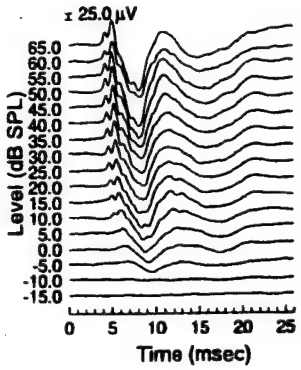

Figure 1. Typical inferior colliculus evoked potential waveforms obtained from a normal chinchilla. Stimulus frequency was 8 kHz. Threshold is −7.5 dB SPL.

Stored waveforms were analyzed visually to determine thresholds. Threshold (dB SPL re: 20 µPa) was defined as the mid-point between the level at which there was a clear deflection in the waveform and the next lower level at which there was none. For example, if there was a clear response at −5 dB SPL and none at −10 dB SPL, the threshold was recorded as −7.5 dB SPL (see Fig. 1).

CDP measurements were made using a system designed in our lab that utilizes three DSP boards housed in a personal computer, insert earphones (Etymotic ER-2), a low noise probe microphone (Etymotic ER-10B), and custom-built attenuators and amplifiers. One DSP board processes microphone output while the other two generate digital signals (primary tones, f_1 and f_2). The primary tones were generated at a sampling rate of 93 kHz and output through 16 bit D/A converters. The microphone output was routed to a 16 bit A/D converter and digitized at a rate of 31 kHz. A Blackman windowing function was applied to the incoming data stream, and a partial discrete Fourier transform was computed. Frequency components corresponding to the two primary frequencies, the cubic distortion product ($2f_1$-f_2), and the noise floor (f_n = 0.7 CDP) were computed. A calibration measurement preceded each input/output (I/O) function, in which the primary tones were presented at an attenuation of 20 dB and the output levels at the primary frequencies were measured and used as reference levels. I/O

functions were collected for primary tones (f_2 = 1.2, 2.4, 3.6, 4.8, 7.2, 9.6, and 12 kHz; f_2/f_1 = 1.2) from 0 to 70 to 80 dB SPL in 5 dB steps. CDP tests followed IC-EVP testing.

Noise Exposures and Acoustic Calibration

The impulse noise was a modified Friedlander wave (0.8 msec A-duration), with a time-amplitude profile simulating impulses created by 5.56 mm rounds fired from a U.S. Army M16A1 rifle (Danielson, Henderson, Gratton, Bianchi, & Salvi, 1991). The digital signal was converted to analog by a D/A converter on a DSP board, attenuated (HP 350D), amplified (NAD 2200), and routed in parallel to two compression drivers (JBL 2446) coupled to sound delivery tubes (5 cm diameter × 20 cm). The ends of the sound delivery tubes were cut at 45° angles to broaden the range of the tube's resonance (Danielson et al., 1991). The drivers faced each other, with the sound delivery tubes separated by 10 cm. Acoustic foam wedges surrounded the drivers to minimize reverberation. An animal was placed in a restraint tube in the 10 cm space between the opposing sound tubes, and 50 pairs of impulses (100 total) were delivered simultaneously to both ears. Impulses in each pair were spaced 50 msec apart, and there was a 1000 msec interval between the onset of each pair (Henselman et al., 1994). The duration of the exposure was therefore less than one minute for each animal.

All exposures were conducted in a 1.8 m × 2.0 m sound booth (Acoustic Systems), where animals were exposed individually. A 1/8" microphone (Bruel & Kjaer Model 4138) was used for acoustic calibration of the impulse noise. The voltage corresponding to a 114 dB tone produced by a pistonphone coupled to the microphone was determined, and used to calculate the desired voltage for a 150 dB peak SPL signal. The attenuation of a manual attenuator (Hewlett Packard 350D) was adjusted to produce the desired signal voltage.

Test Schedule after Noise Exposure

IC-EVPs and CDPs were measured from impulse noise-exposed animals at 15 minutes, 24 hr, and 5 days postexposure to monitor TTS, and after 25 to 35 days recovery from exposure to determine PTS. Before exposure, each animal was tested three times, and the average of the three measurements was used as the stable baseline estimate of sensitivity. Threshold shifts (TSs) of each animal were calculated by subtracting mean pre-exposure IC-EVP thresholds from postexposure thresholds. After 25 to 35 days recovery from high-level exposure, IC-EVPs and CDPs were measured on three separate occasions and averaged to calculate PTS at each frequency.

Cochlear Histology

At the end of testing, chinchillas were deeply anesthetized with sodium pentobarbital (Somlethal, 100 mg/kg i.p.) and decapitated. The cochleas were quickly removed and perfused through the oval window with a succinate dehydrogenase staining solution (2.5 mL, 0.2 M sodium succinate, 2.5 mL, 0.2 M phosphate buffer, pH 7.6, and 5 mL, 0.1% tetranitro blue tetrazolium). Cochleas were then incubated in the succinate dehydrogenase staining solution for 45 minutes at 37° C, postfixed with 10% formalin, and stored in fixative. Stained cochleas were dissected from the apex to the base, mounted in sections in glycerin on microscope slides, coverslipped, and examined using light microscopy (400× magnification). Percent hair cells missing was referenced to our lab standards based on average hair cell counts from nine cochleas of young (<1 yr old), healthy chinchillas.

Data Analyses

Data were obtained from both ears of 37 animals (19 male, 18 female) and from a single ear of 36 animals, so that the final sample for data analysis consisted of 110 ears (55 male, 55 female). Noise-exposure data were obtained from 28 ears (15 male, 13 female). Data analyses were geared toward answering the following questions: 1) Are there significant sex differences in auditory sensitivity, IC-EVP amplitudes, or CDP I/O functions? 2) Are there sex differences in TTS and/or PTS caused by exposure to simulated M16 rifle fire? Analyses of variance (ANOVAs) were used to assess differences between means. The dependent variables were IC-EVP thresholds and IC-EVP threshold shifts (TSs) at various times after noise exposure. Independent variables were Sex (a between-subjects factor), Frequency and Time of Assessment (within-subjects factors). Significant main effects and interactions involving Sex were analyzed further using 1-way ANOVAs or independent Student *t*-tests. Within a group, changes as a function of time or frequency were assessed using paired *t*-tests. Mean IC-EVP and CDP amplitude functions for females and males were compared by calculating the 95% confidence interval for the difference between the means. All statistical tests were evaluated using a 0.05 criterion of significance.

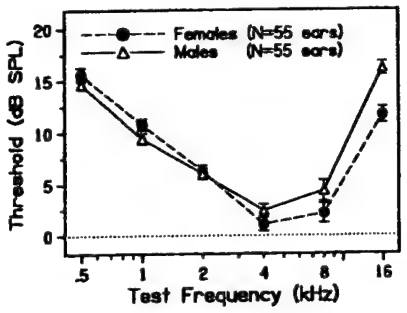

Figure 2. Pre-exposure thresholds of female (solid circles) and male (open triangles) chinchillas. Differences between females and males were statistically significant at 16 kHz only. Bars represent standard errors of the means.

RESULTS

Basic Auditory Sensitivity of Female and Male Chinchillas

IC-EVP Thresholds • Thresholds of female and male chinchillas are shown in Figure 2. As a group, male chinchillas have slightly lower thresholds than females at frequencies below 2 kHz, whereas female chinchillas have slightly lower thresholds than males at frequencies above 2 kHz. The differences are generally small, but consistent.

A 2-way mixed ANOVA, with Sex as a between-subjects factor and Frequency as a within-subjects factor, revealed a significant Sex × Frequency interaction, $F(5, 540) = 7.58$; $p < 0.001$. Follow-up analyses indicated that mean threshold at 16 kHz was significantly higher for males than for females (16.15 ± 4.8 dB versus 11.64 ± 5.7 dB), $F(1, 108) = 20.24$; $p < 0.0001$. Thresholds at frequencies below 16 kHz were not significantly different between the two sexes.

IC-EVP and CDP Amplitude Functions • Mean IC-EVP I/O functions at 0.5, 1, 2, 4, 8, and 16 kHz are shown in Figure 3. The thin lines represent means for female chinchillas, and the hatched regions surrounding them represent the 95% confidence intervals. The thick lines represent the means for male chinchillas. It is apparent from Figure 3 that there were no significant sex differences in mean I/O functions despite slight differences in IC-EVP thresholds (see Fig. 2).

Similarly, there were no meaningful differences between male and female chinchillas in their CDP I/O functions (Fig. 4). The thin lines in Figure 4 represent means for females, and the hatched regions surrounding them represent the 95% confidence intervals. The thick lines represent means for males. The CDP frequency is indicated above each panel. CDP I/O functions were very similar for males and females, with thresholds around 20 to 30 dB SPL at all frequencies, and amplitudes increasing monotonically over the entire range of input

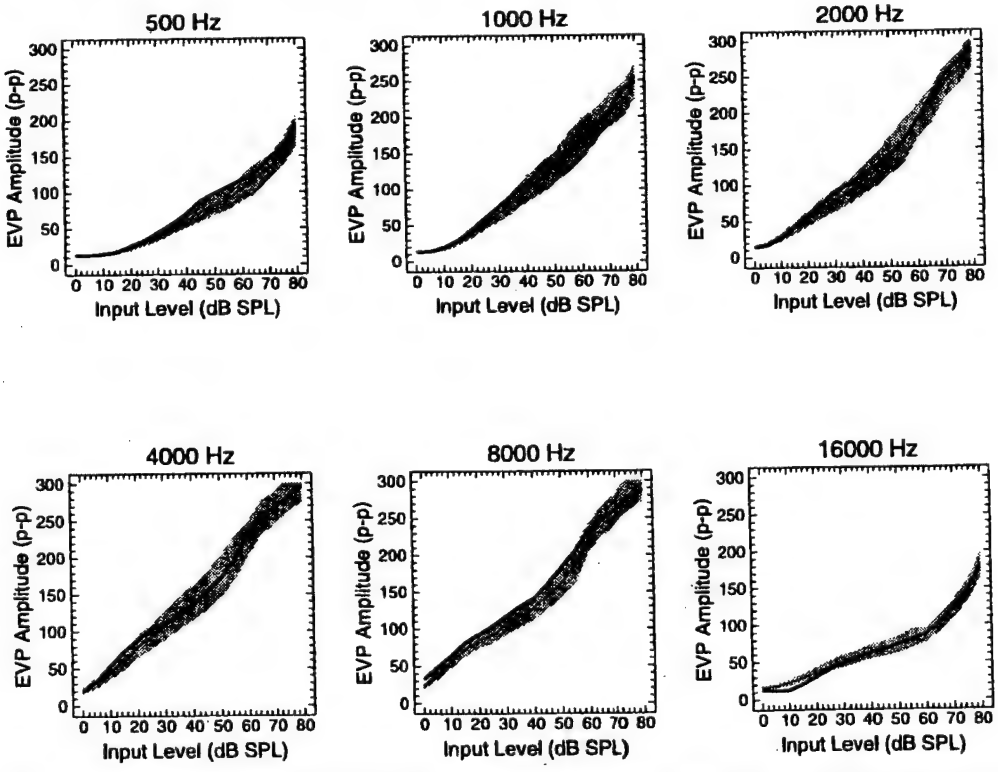

Figure 3. Pre-exposure inferior colliculus evoked potential input/output functions for female (thin line) and male (thick lines) chinchillas. Hatched regions represent the 95% confidence interval for the difference between means. EVP = evoked potential.

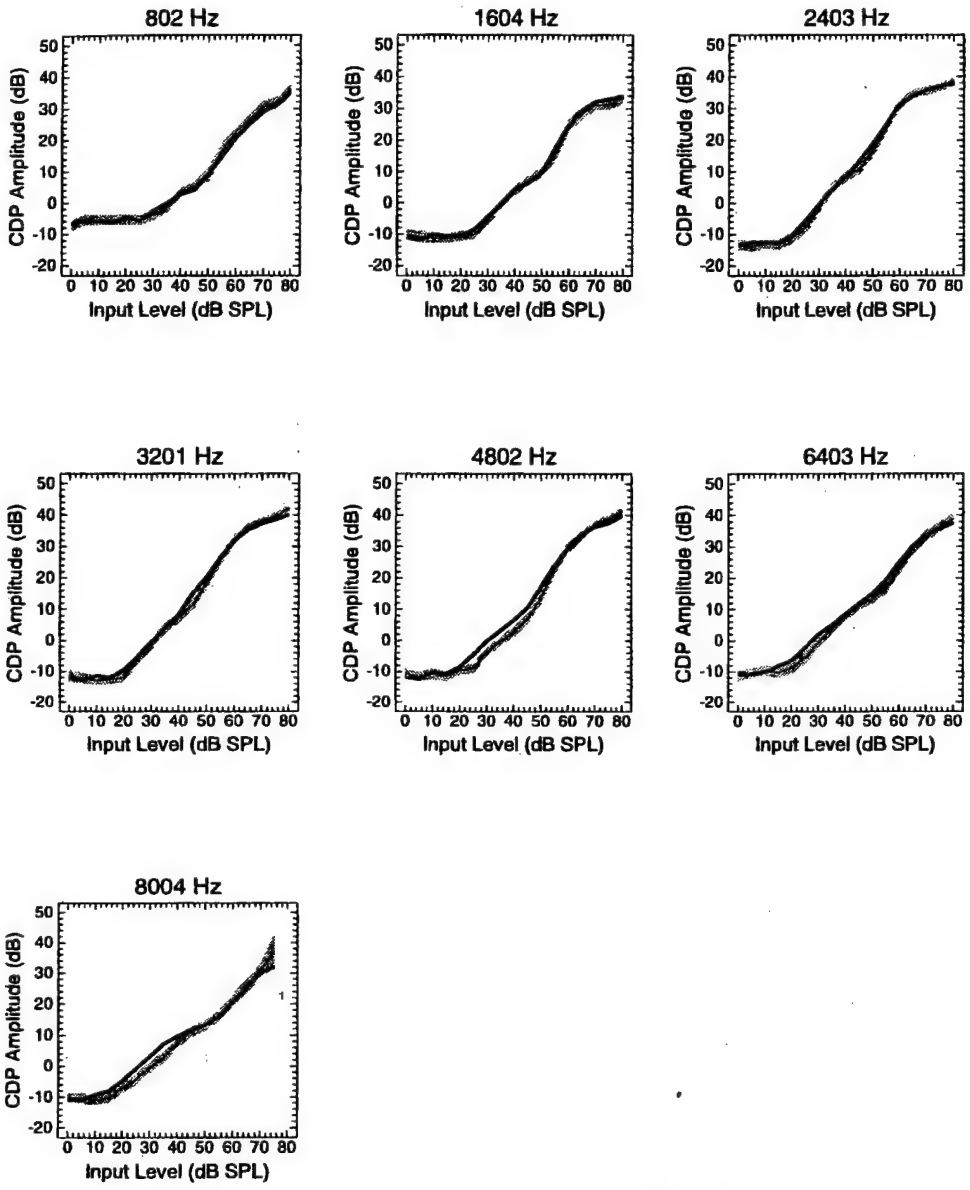

Figure 4. Pre-exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (CDP) input/output functions for female (thin line) and male (thick lines) chinchillas. Hatched regions represent the 95% confidence interval for the difference between means. The parameter above each panel indicates the CDP frequency ($2f_1$-f_2).

levels. Overall, the results indicate that there are no meaningful sex differences in amplitudes of either IC-EVPs or CDPs before noise exposure, despite small differences in thresholds (Fig. 2).

Sex Differences in Hearing Loss from Simulated M16 Rifle Fire

Pre-Exposure Thresholds • Pre-exposure IC-EVP thresholds for the subset of animals exposed to noise are shown in Figure 5. Although females exhibited slightly lower thresholds than males at several frequencies, particularly at 8 and 16 kHz, a 2-way (Sex × Frequency) mixed ANOVA did not detect significant differences between the sexes.

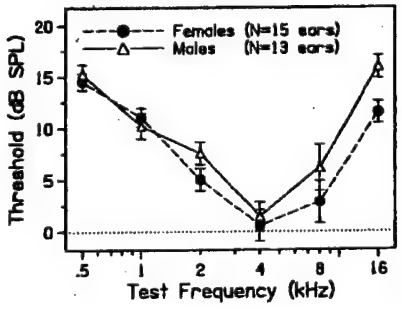

Figure 5. Pre-exposure thresholds of the female (solid circles) and male (open triangles) chinchillas that were subsequently exposed to 150 dB pSPL impulse noise. Bars represent standard errors of the means.

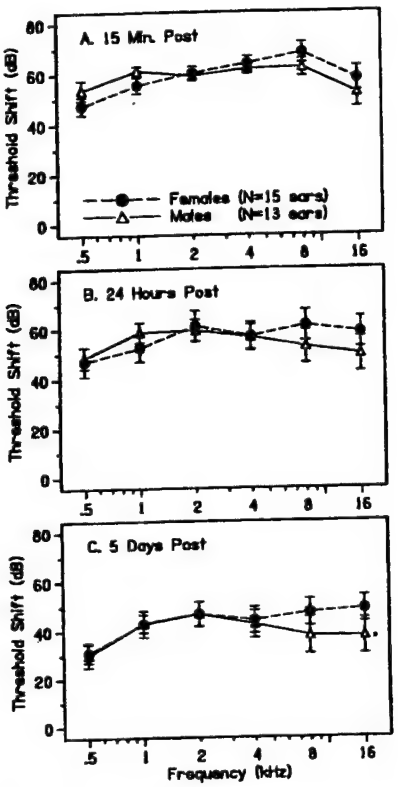

Figure 6. Threshold shifts of female (solid circles) and male (open triangles) chinchillas at 15 minutes, 24 hr, and 5 days after exposure to 150 dB pSPL impulse noise. Bars represent standard errors of the means.

Postexposure TS • Mean IC-EVP TSs measured at 15 minutes, 24 hr, and 5 days after exposure to 150 dB peak SPL impulse noise are shown in Figure 6. When tested 15 minutes after the high-level exposure, both females and males exhibited significant threshold elevations (47 to 68 dB) at all frequencies (Fig. 6a). Males showed approximately 5 to 6 dB more TS than females at 0.5 and 1 kHz, whereas females showed approximately 6 dB more TS than males at 8 and 16 kHz. A 2-way mixed (Sex × Frequency) ANOVA indicated that there was a significant Sex × Frequency interaction, $F(5, 130) = 2.1$; $p^* = 0.05$, but no main effect of Sex. Follow-up analyses indicated that TS increased progressively from 0.5 kHz to 8 kHz for females. Differences between successive frequencies were significant from 0.5 kHz to 4 kHz; TS was equivalent at 4 and 8 kHz, then declined significantly between 8 and 16 kHz (all p values < 0.01). In contrast, males showed a much flatter pattern of TS, with statistically equivalent TS at 1, 2, 4, and 8 kHz.

Relatively little threshold recovery occurred between 15 minutes and 24 hr postexposure. Females showed TS decreases of 0 to 7 dB, and males showed TS decreases of 0 to 9 dB. Both females and males showed the greatest recovery (5 to 9 dB) at 4 and 8 kHz. As shown in Figure 6b, TS ranged from approximately 47 dB at 0.5 kHz to 61 dB at 8 kHz. Females exhibited approximately 9 dB more TS than males at 8 and 16 kHz, and approximately 5 dB less TS than males at 1 kHz. A 2-way ANOVA yielded a significant Sex × Frequency interaction, $F(5, 130) = 2.4$; $p = 0.044$. Whereas females had statistically equivalent TS at 2, 4, 8, and 16 kHz and significantly less TS at 0.5 and 1 kHz, males had equivalent TS at 1, 2, 4, and 8 kHz, and significantly less TS at 0.5 and 16 kHz than at intermediate frequencies.

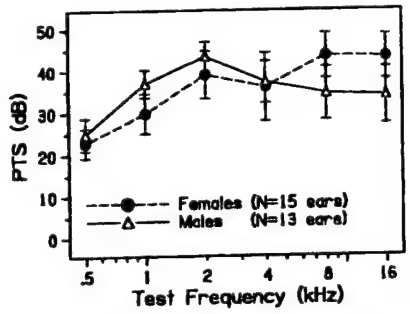

Figure 7. Permanent threshold shifts (PTSs) of female (solid circles) and male (open triangles) chinchillas, measured 30 days after exposure to 150 dB pSPL impulse noise. Bars represent standard errors of the means.

Significant recovery occurred between 1 and 5 days after exposure, with TS decreasing by 9 to 19 dB. Females and males showed equivalent TS at frequencies ≤4 kHz, whereas females exhibited approximately 9 dB and 11 dB more TS than males at 8 and 16 kHz, respectively (Fig. 6c). However, the 2-way (Sex × Frequency) ANOVA did not reveal any significant differences between the sexes at this time.

Mean thresholds improved by 4 to 13 dB between 5 and 30 days postexposure, when permanent hearing loss was assessed (Fig. 7). High-level exposure produced significant PTS at all frequencies for both females and males (paired t-tests; all p values $<$ 0.001). PTS ranged from 23 to 43 dB, with females showing 2 to 7 dB less PTS than males at low frequencies (0.5 to 2 kHz), and approximately 9 dB more PTS at 8 and 16 kHz. A significant Sex × Frequency interaction was obtained, $F(5, 130) = 3.10$; $p = 0.011$. Follow-up analyses indicated that both males and females developed progressively and significantly greater PTS from 0.5 to 2 kHz. However, PTS peaked at 2 kHz for males, and declined significantly at higher frequencies. Females, in contrast, had significantly greater PTS at 8 and 16 kHz than at lower frequencies.

CDP amplitude data are generally consistent with the IC-EVP data. Before noise exposure, CDP I/O functions were similar for males and females, as

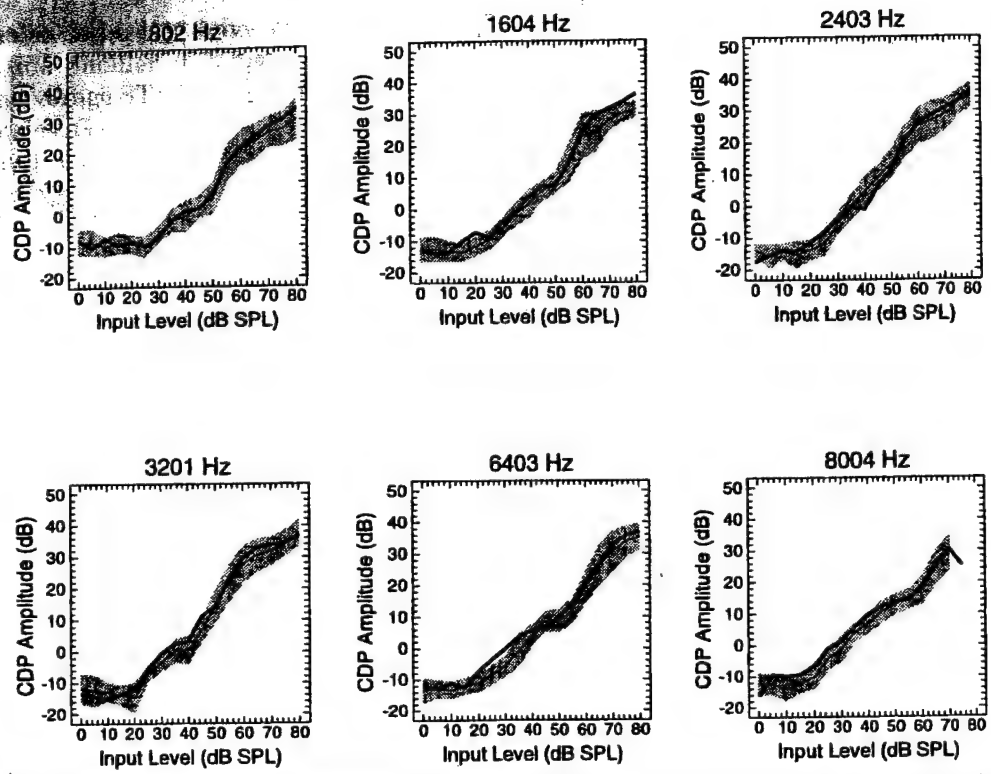

Figure 8. Cubic ($2f_1$-f_2) distortion product otoacoustic emission (CDP) input/output functions for female (thin line) and male (thick lines) chinchillas before noise exposure. Hatched regions represent the 95% confidence interval for the difference between means. The parameter above each panel indicates the CDP frequency ($2f_1$-f_2).

shown in Figure 8. After exposure, however, CDP amplitudes were significantly depressed (Fig. 9). There was a trend for males to have lower amplitude CDPs than females at low frequencies (where males had greater PTS) but higher CDP amplitudes at high frequencies (where males had less PTS).

Hair Cell Losses • Sixteen cochleas (eight female, eight male) were examined for hair cell losses. As shown in Figure 10, outer hair cell (OHC) loss (left panel) exceeded inner hair cell (IHC) loss (right panel), with OHC losses ranging from 70 to 100% in the basal half of the cochlea for both sexes. Males sustained substantially greater IHC and OHC losses than females. IHC losses for males peaked in the 2 to 3 kHz region of the cochlea, with an average loss of approximately 80%. In contrast, average IHC losses for the females did not exceed 30% in any region of the cochlea. OHC losses of females were approximately 20% less than OHC losses of males in the cochlear regions (>1 kHz) where OHC loss occurred.

DISCUSSION

The results indicate that female and male chinchillas differ slightly in their basic auditory sensitivity, with females tending to have lower thresholds at high frequencies and higher thresholds at low frequencies. More importantly, the results point to a fundamental sex difference in the response of the chinchilla cochlea to high-level impulse noise. Female chinchillas sustained more high-frequency hearing loss, less low-frequency hearing loss, and less hair cell loss than males. The reasons for the sex differences observed both before and after noise exposure cannot be determined from this study. However, because the differences were observed in chinchillas, they cannot be attributed to differences in noise exposure history, recreational activities, dietary factors, or other extraneous variables that complicate interpretation of gender differences in humans (Henderson, Subramaniam, & Boettcher, 1993). Therefore, the data from the chinchilla may be particularly useful in interpreting findings from previous studies with humans.

Small but consistent gender differences in auditory sensitivity have been well documented in humans (e.g., Chung et al., 1983; Corso, 1963; Pearson et al., 1995; Ward, 1966). In general, females tend to have slightly lower pure-tone thresholds than males at frequencies above 1 to 2 kHz, whereas males may have slightly lower thresholds below 1 to 2 kHz. Chung et al. (1983) analyzed data from more than 50,000 people and found that the average difference between males and females in hearing sensitivity was 2 to 3.5 dB for test frequencies above 2 kHz, and

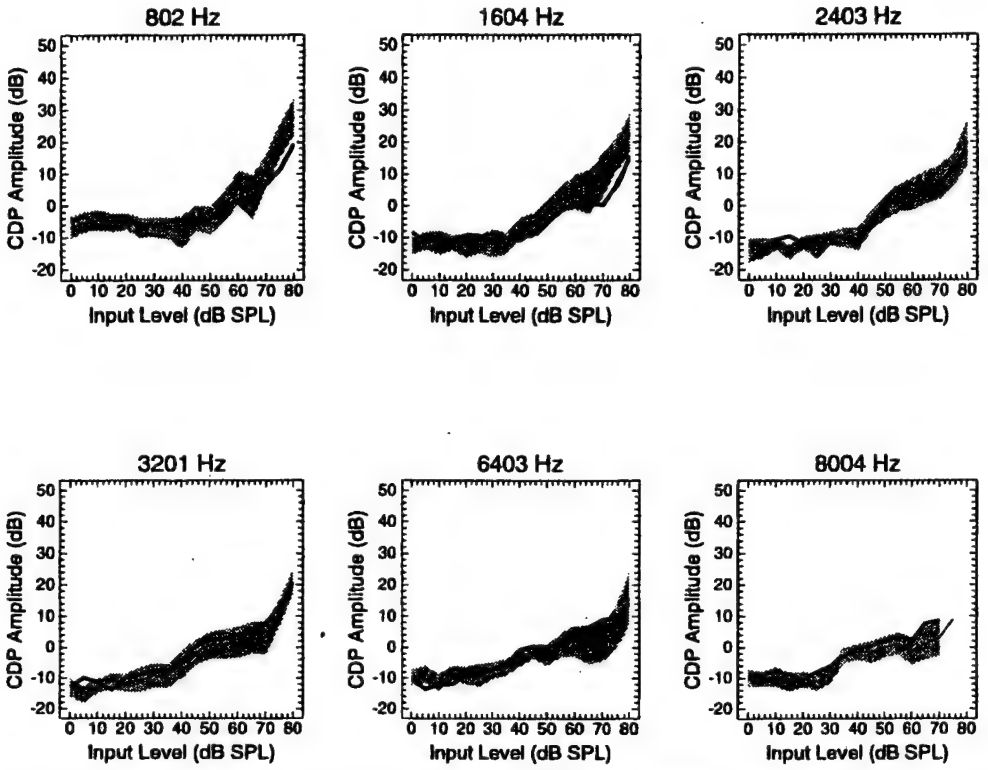

Figure 9. Cubic ($2f_1$-f_2) distortion product otoacoustic emission (CDP) input/output functions for female (thin line) and male (thick lines) chinchillas 30 days after noise exposure. Hatched regions represent the 95% confidence interval for the difference between means. The parameter above each panel indicates the CDP frequency ($2f_1$-f_2).

less than 1 dB for frequencies at or below 2 kHz. Ward (1966) found that thresholds of young adult females were up to 6 dB better than thresholds of young adult males at frequencies above 2.8 kHz. Although differences in auditory sensitivity have sometimes been attributed to gender-related differences in noise exposure history, the current data from chinchillas argue for inherent anatomical and/or physiological differences between the sexes. Recently, Pearson et al. (1995) reported the results of the Baltimore Longitudinal Study of Aging, which tracked thresholds of 681 men and 416 women in low-noise occupations who were screened for otological disorders and NIHL. Their results provide further evidence of small gender differences in thresholds while ruling out occupational noise exposure as the cause for poorer thresholds in men. Women had significantly better thresholds than men at all frequencies above 1 kHz, whereas men had better thresholds at 0.5 kHz, and men and women did not differ at 1 kHz.

Sex/gender differences in both basic sensitivity and in susceptibility to NIHL could arise from differences in the acoustical properties of the outer and middle ears. In a recent study, Hellstrom (1995b) showed a significant relationship between the sound transfer function (STF) of the external ear, ear canal dimensions, and hearing levels in male and female

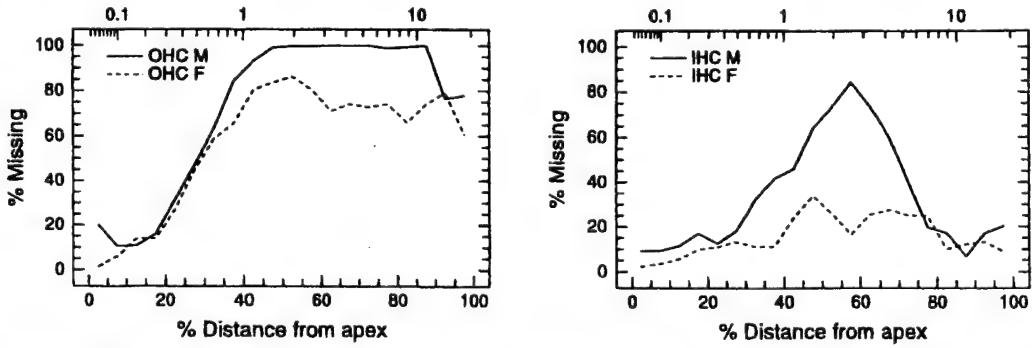

Figure 10. Mean hair cell losses after impulse noise exposure. Left panel: outer hair cell (OHC) loss; right panel: inner hair cell (IHC) loss.

subjects. Females tended to have ear canals that were shorter and smaller in volume than males, resulting in an average STF that was shifted toward higher frequencies. Gender differences in the STF of the external and middle ears would be expected to influence susceptibility to NIHL as well as basic auditory sensitivity (Hellstrom, 1995a, b; Saunders & Tilney, 1982; Tonndorf, 1976).

We are not aware of any published studies of sex differences in ear canal characteristics in nonhuman species. Consequently, the possibility that the sex differences observed in the present study are due to systematic differences in STFs cannot be ruled out. However, several factors suggest that the STF is not the sole basis for sex/gender differences in chinchillas or humans. First, data presented by Saunders and Tilney (1982) show that the chinchilla ear canal STF is a sharply peaked function, with gain increasing from approximately 5 dB SPL at 4.8 kHz to 23 dB at 10 kHz, then dropping to 5 dB around 14 kHz. This STF contrasts with the human ear canal STF, which has a resonant peak between 2 and 4 kHz (Hellstrom, 1995b), yet the pattern of sex/gender differences for chinchillas and humans are quite similar. Hellstrom himself noted that certain aspects of his data were difficult to account for in terms of STF. In particular, there is no obvious reason why subjects with elevated STFs at 4 kHz tended to have lower thresholds at 2 kHz than subjects with elevated STFs at 2 kHz.

A second point to consider is that there are numerous gender differences that are not easily accounted for by the STF. Gender differences have been observed in 1) the upper limit for perceiving binaural beats (Tobias, 1965), with women having a significantly lower cutoff frequency than men (600 versus 800 Hz); 2) the incidence of spontaneous otoacoustic emissions, with women exhibiting them significantly more often than men (Bell, 1992; Bilger, Matthies, Hammel, & DeMorest, 1990; Whitehead, Baker, & Wilson, 1989); and 3) auditory brain stem responses, with women having shorter central conduction times, even after differences in head size are taken into account (Edwards, Squires, Buchwald, & Tanguay, 1983; Patterson, Michalewski, Thompson, Bowman, & Litzelman, 1981; Trune, Mitchell, & Phillips, 1978). Gender differences such as these suggest that factors other than simple acoustics may be involved. Third, studies have reported that hearing sensitivity (Baker & Weiler, 1977; Cox, 1980; Davis & Ahroon, 1982; Miller & Gould, 1967; Swanson & Dengerink, 1988), spontaneous otoacoustic emissions (Bell, 1992; Penner, 1995), auditory brain stem responses (Elkind-Hirsch, Stoner, Stach, & Jerger, 1992), and susceptibility to TTS (Davis & Ahroon, 1982; Dengerink et al., 1984; Petiot & Parrot, 1984) can all fluctuate in monthly cycles in women, or differ between normally cycling women and women taking oral contraceptives. The above considerations suggest that factors other than (or in addition to) the STF may be responsible for sex/gender differences in basic auditory sensitivity and susceptibility to NIHL. Future studies using the chinchilla may help determine the relative importance of anatomical and physiological factors in sex/gender differences in auditory sensitivity and susceptibility to NIHL.

ACKNOWLEDGMENTS:

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Association for Research in Otolaryngology

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Tracking Id: 4631

Abstract Type: Contributed Abstract

Track: Poster

Session: Other

Title: Systemic treatment with estradiol reduces noise-induced hearing loss in the chinchilla

Author Information: Daniel Lockwood; Sandra MCFADDEN; Haiyan Jiang; Lisa Rosenberg

Submitted By: Sandra MCFADDEN

Sponsor: MCFADDEN , Sandra

Abstract Text: The effects of estrogen (E) on noise-induced hearing loss were investigated in two experiments. Chinchillas were prepared for evoked potential recording by implanting electrodes into each inferior colliculus and the rostral cranium. Evoked potential thresholds were measured prior to noise exposure and at 15 min, 24 hr, 7 days and 14 days after exposure to 50 sec of impulse noise (Experiment I) or 4 hr of continuous noise (Experiment II). After baseline measurements were obtained, animals were randomly assigned to E or control groups. Animals in E groups received daily subcutaneous injections of 17-Beta estradiol (Sigma Chemicals) dissolved in olive oil. Animals in a vehicle control group received subcutaneous injections of olive oil on the same schedule as E animals. Animals in a separate control group received no treatment. In Experiment I, 10 animals received 200-265 mg E for 1-2 weeks prior to exposure. Evoked potential thresholds were not affected by E treatment. Following exposure to 50 pairs of impulses at 150 dB peak SPL, the E group showed significantly less permanent threshold shift as compared to vehicle controls (N=4). In Experiment II, animals received either 100 mg (N=5) or 725 mg (N=6) E for 1 week prior to exposure. Following exposure to octave band noise centered at 4 kHz at 105 dB SPL, the E group showed significantly less permanent threshold shift as compared to controls, and the high-dose group had less hearing loss than the low-dose group. The protective effects of E could be related to its antioxidant properties, its modulatory effects on neurotransmitter function, or to other properties of the steroid hormone.

Session Topic: Alternate Session Inner Ear: Abnormal Structure or Function

Equipment:

Equipment	Quantity

Differences Between Female and Male Chinchillas in Susceptibility to Noise-Induced Hearing Loss

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ABSTRACT

Small gender differences in auditory sensitivity have been well documented in humans. In addition, experimental studies of temporary threshold shifts (TS) and retrospective studies of hearing loss in industrial workers suggest that males are more susceptible to TS from low frequency exposures, whereas females are more susceptible to TS from high-frequency exposures. Whether these differences are due to differences in noise exposure history, diet, and noise induced stress levels or to inherent anatomic and physiological factors has not been determined. Furthermore, there is no information regarding gender differences in susceptibility to impulse noise. We conducted 6 separate experiments in which evoked potential thresholds were obtained from female and male chinchillas before and after exposure in vivo. Each experiment used a different noise exposure condition: (1) 4 kHz octave band noise (OBN) at 105 dB SPL for 4 hr; (2) impulse noise (simulated M16 rifle fire) at 150 dB pSPL with (a) a single driver positioned in front of the animal and (b) two drivers facing each ear and firing simultaneously; (3) 0.5 kHz OBN at 90-95 dB SPL for 5 days, followed by impulse noise; (4) noise simulating an Army Blackhawk helicopter at (a) 90 dB SPL for 5 days, followed by impulse noise, and (b) 112 dB SPL for 5 days followed by impulse noise. The results show that prior to exposure, female chinchillas tend to have four or low-frequency thresholds and lower high frequency thresholds than males. The differences are generally small (less than 5 dB SPL), but consistent across experiments. After exposure, males showed more low-frequency hearing loss (up to 15 dB) than females in all experiments. Females showed more high frequency hearing loss (up to 20 dB) than males in 4 experiments. Cochleograms generally showed greater hair cell losses in males than in females. Overall, the results suggest that there are sex differences in basic auditory sensitivity and in the physiological and anatomic responses of the chinchilla cochlea to damaging levels of noise. Future studies with the chinchilla may be useful for understanding the nature of gender differences in humans.

Supported by Grant DAMD17-96-1-6338 to SLM.

INTRODUCTION

Previous studies[1-5] have reported small but consistent gender differences in basic auditory sensitivity and susceptibility to noise-induced hearing loss (NIHL). In general, females tend to have slightly better pure-tone thresholds than males at frequencies above 1-2 kHz, while males may have slightly better thresholds below 1-2 kHz. Experimental studies have shown that males exhibit more temporary hearing loss (TTS) than females from low-frequency exposures (below 2 kHz), whereas females exhibit more TTS than males from high-frequency exposures (above 2 kHz). In an early investigation of gender differences in susceptibility to TTS produced by high intensity tones and noise, Ward (1966) conducted 17 experiments with 24 male and 25 female adults. Females showed approximately 30% less TTS than males when the exposure frequency was below 1 kHz, but approximately 30% more TTS when the exposure frequency was above 2 kHz. Most of what little we know about gender differences in permanent hearing loss (PTS) comes from retrospective studies of workers exposed to noise in industrial settings. Under these conditions, which typically involve exposure to low-frequency continuous noises, males tend to develop much more hearing loss (approximately 20 dB) than females.

The reasons for gender differences in auditory sensitivity and susceptibility to NIHL are unknown, but are generally attributed to systematic differences in the physical characteristics of the outer, middle and inner ears and/or noise exposure history. There have been no studies of sex differences in susceptibility to NIHL in non-human species to shed light on the issue. Here, we present data from six separate experiments conducted with chinchillas that suggest there are fundamental differences between female and male chinchillas in their auditory sensitivity and susceptibility to NIHL.

METHODS

All procedures were reviewed and approved by the University of Buffalo Animal Care and Use Committee, and conformed to federal guidelines for the humane treatment of laboratory animals.

Subjects and Surgery Chinchillas between 1 and 3 years of age were used. Recording electrodes were implanted in the left and/or right inferior colliculus (IC), and in the rostral cranium.

Test Procedures Evoked potentials were recorded from the implanted electrodes before and 20-30 days after exposure to noise. The awake chinchilla was placed in a restraining device that held its head at a constant orientation within the calibrated sound field of a sound-attenuating booth. Seventy-seven 10 ms tones (2 ms rise/fall, 20/s rate) at octave intervals from 0.5 to 16 kHz. Threshold (dB SPL re: 20 μPa) was defined as the mid-point between the level at which there was a clear deflection in the waveform and the next lower level (5 dB step size) at which there was none.

Noise exposures The noises were: (1) 4 kHz octave band noise (OBN) at 105 dB SPL for 4 hr (Exp. 1); (2) impulse noise simulating M16 rifle fire at 150 dB peak SPL (50 pairs impulses in a pair spaced 50 ms apart, 1000 ms interval between the onset of each pair; 50 ms rise/fall time) using (a) a single driver positioned in front of the animal (Exp. 2) or (b) two loudspeaker drivers facing each ear and firing simultaneously (Exp. 3); (3) 0.5 kHz OBN at 90-95 dB SPL for 5 days, followed by impulse noise (Exp. 4); (4) noise simulating UH60 Black Hawk helicopter noise at (a) 90 dB SPL for 5 days, followed by impulse noise (Exp. 5) or (b) 112 dB SPL for 10 days, followed by impulse noise (Exp. 6). Experiments 3-6 utilized two drivers for the impulse noise, and a 5-day (experiment 5) or 10-day (experiment 6) recovery period separated the two noise exposures. The spectra of the noises are shown in Figure 1.

Cochlear Analyses Cochleas were stained with a succinate dehydrogenase (SDH) staining solution and post-fixed with 10% formalin. Surface preparations of the organ of Corti were examined with light microscopy. Cochleograms were constructed to show the percent of inner hair cells (IHCs) and outer hair cells (OHCs) missing as a function of location on the basilar membrane. In addition, mean cochlear length was determined for each group.

RESULTS

EXP. 1. 4 kHz OBN, 105 dB SPL, 4 Hours

Fig. 1. Pre-exposure thresholds and PTS of 4 females (8 ears) and 2 males (4 ears). Two-way mixed ANOVAs detected significant main effects of Frequency for both pre-exposure thresholds ($F(5,50)=21.25$, $p<0.001$) and PTS ($F(5,50)=10.97$, $p<0.001$).

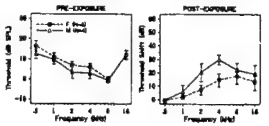
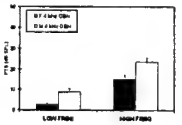


Fig. 2. Mean low-frequency (0.5, 1 and 2 kHz) versus high-frequency (4, 8 and 16 kHz) PTS.



EXP. 2. Impulse Noise, 150 dB pSPL, 50 pairs, 1 Driver

Fig. 3. Pre-exposure thresholds and PTS of 2 females (4 ears) and 5 males (6 ears). Two-way mixed ANOVAs detected significant main effects of Frequency ($F(5,55)=22.31$, $p<0.001$) for pre-exposure thresholds, and of Sex ($F(1,11)=6.48$, $p=0.027$) and Frequency ($5,55)=5.34$, $p<0.001$) for PTS.

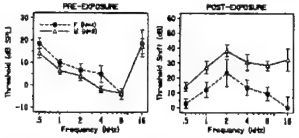


Fig. 4. Noise-induced OHC loss (left panel) and IHC loss (right panel). Mean cochlear length was 19.08 ± 0.4 for females, and 18.91 ± 0.8 for males.

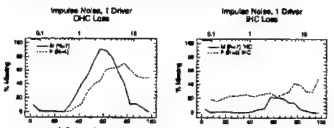
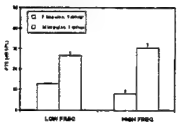


Fig. 5. Mean low-frequency versus high-frequency PTS.



EXP. 3. Impulse Noise, 150 dB pSPL, 50 pairs, 2 Drivers

Fig. 6. Pre-exposure thresholds and PTS of 9 females (15 ears) and 9 males (15 ears). Two-way mixed ANOVAs detected significant main effects of Frequency ($F(5,140)=47.07$, $p<0.001$) for pre-exposure thresholds, and of Sex ($F(1,11)=6.48$, $p=0.027$) and Frequency ($F(1,28)=8.13$, $p=0.02$) for PTS.

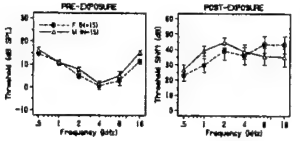
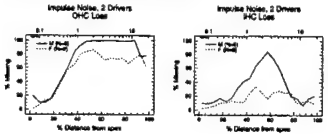


Fig. 7. Noise-induced OHC loss (left panel) and IHC loss (right panel). Mean cochlear length was 18.75 ± 0.8 for females, and 18.23 ± 0.6 for males.



EXP. 4. 0.5 kHz OBN, 90 dB SPL, 5 Days + Impulse Noise

Fig. 8. Pre-exposure thresholds and PTS of 6 females (11 ears) and 7 males (13 ears). Two-way mixed ANOVAs detected significant Sex X Frequency interactions for both pre-exposure thresholds ($F(5,110)=2.62$, $p=0.028$) and PTS values ($F(5,110)=4.20$, $p=0.002$), as well as main effects of Frequency ($F(5,110)=36.34$, $p<0.001$ for pre-exposure thresholds, and $F(5,110)=8.45$, $p<0.001$ for PTS).

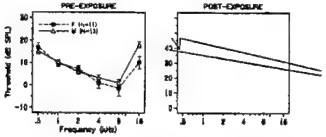
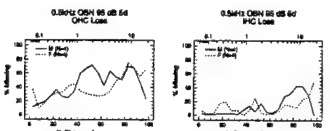


Fig. 9. Noise-induced OHC loss (left panel) and IHC loss (right panel). Mean cochlear length was 17.76 ± 0.6 for females, and 19.02 ± 0.6 for males.



EXP. 5. UH60 Helicopter Noise, 90 dB SPL, 5 Days + Impulse Noise

Fig. 10. Pre-exposure thresholds and PTS of 6 females (12 ears) and 6 males (12 ears). Two-way mixed ANOVAs detected significant Sex X Frequency interactions for both pre-exposure thresholds ($F(5,110)=2.36$, $p=0.045$) and PTS ($F(5,110)=2.45$, $p=0.038$), as well as significant main effects of Frequency ($F(5,110)=86.63$, $p<0.001$ for pre-exposure thresholds, and $F(5,110)=16.27$, $p<0.001$ for PTS).

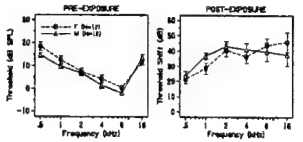
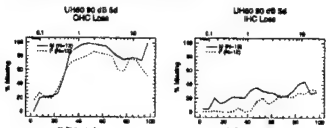


Fig. 11. Noise-induced OHC loss (left panel) and IHC loss (right panel). Mean cochlear length was 18.73 ± 1.2 for females, and 18.43 ± 0.5 for males.



EXP. 6. UH60 Helicopter Noise, 112 dB SPL, 10 Days + Impulse Noise

Fig. 12. Pre-exposure thresholds and PTS of 8 females (8 ears) and 6 males (6 ears). Two-way mixed ANOVAs detected significant Sex X Frequency interactions for both pre-exposure thresholds ($F(5,80)=2.69$, $p=0.019$) and PTS ($F(5,80)=2.61$, $p=0.004$), as well as main effects of Frequency ($F(5,80)=26.61$, $p<0.001$ for pre-exposure thresholds, and $F(5,80)=13.20$, $p<0.001$ for PTS).

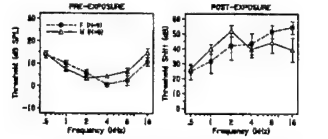


Fig. 13. Noise-induced OHC loss (left panel) and IHC loss (right panel). Mean cochlear length was 18.00 ± 0.9 for females, and 18.96 ± 0.7 for males.

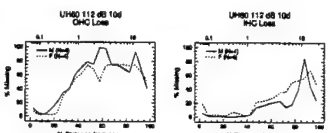


Fig. 14. Summary of Low-Frequency and High-Frequency PTS for Experiments 3-6.

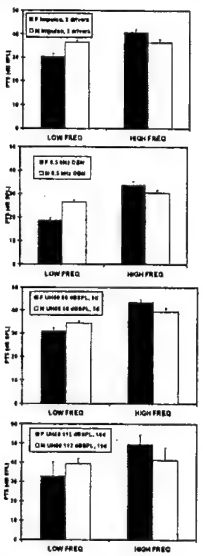
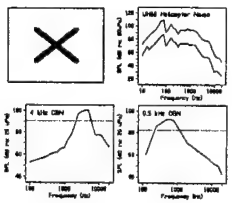


Fig. 15. Mean Length of Cochleas

Fig. 16. Noise Spectra (1/3 Octave Band Level Measurements)



SUMMARY AND CONCLUSIONS

Female and male chinchillas differ slightly in their basic auditory sensitivity, with females tending to have lower thresholds at high frequencies and higher thresholds at low frequencies. More importantly, the results point to a fundamental sex difference in the response of the chinchilla cochlea to noise. Female chinchillas sustained more high-frequency hearing loss in 4/6 experiments, less low-frequency hearing loss in 6/6 experiments, and less hair cell loss in 6/6 experiments. The reasons for the sex differences cannot be determined from this study. However, since the differences were observed in chinchillas, they cannot be attributed to differences in noise exposure history, recreational activities, dietary factors, or other extraneous variables that complicate interpretation of gender differences in humans. Our measurements of cochlear length indicate that sex differences in susceptibility to NIHL are also not related to differences in the length of the cochlea in chinchillas.

Sex/gender differences in both basic sensitivity and in susceptibility to NIHL could arise from differences in the acoustical properties of the outer and middle ears[10-11], or from other as yet unknown physiological differences. Future studies using the chinchilla may help determine the relative importance of anatomical and physiological factors in sex/gender differences in auditory sensitivity and susceptibility to NIHL.

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Conditioning-induced protection from impulse noise in female and male chinchillas

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Abstract

Sound conditioning (pre-exposure to a moderate-level acoustic stimulus) can increase a subject's resistance to hearing loss from a subsequent traumatic exposure. Nearly all sound conditioning experiments have utilized long-duration tones and noise at levels below 110 dB SPL as traumatic stimuli. It is important to know if sound conditioning can also provide protection from brief, high-level stimuli such as impulses produced by gunfire, and whether there are differences between females and males in the ability to develop resistance through sound conditioning. In the present study, chinchillas were exposed to an octave band noise centered at 0.5 kHz for 6 h/day for 5 days. After 5 days of recovery, they were exposed to simulated M16 rifle fire at a level of 150 dB peak SPL. Animals that were sound conditioned showed less hearing loss and smaller hair cell lesions than controls. Females showed less low-frequency hearing loss but more high-frequency hearing loss than males. Cochleograms showed slightly less hair cell loss in females than in males. The results show that significant protection from impulse noise can be achieved with a 5-day conditioning regimen, and that there are consistent differences between female and male chinchillas in the response of the cochlea to impulse noise.

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Key words: sound conditioning, impulse noise, acoustic trauma, gender, auditory evoked potentials, cochlea

INTRODUCTION

Recent studies have shown that pre-exposure to a moderate-level acoustic stimulus ("sound conditioning") can increase a subject's resistance to noise-induced hearing loss (NIHL). The protective effects of sound conditioning were reported first by Canlon et al. (1988), who found that guinea pigs that had been exposed to a 1 kHz tone at 81 dB sound pressure level (SPL re: 20 μPa) for 24 days incurred less permanent threshold shift (PTS) from a subsequent exposure to the tone at 105 dB SPL for 72 h than animals that had not been similarly "trained" or "conditioned." Since then, numerous studies have shown that sound conditioning can provide protection from NIHL in a wide variety of species and across a wide range of conditioning parameters. However, nearly all sound conditioning experiments have used long-duration tones or noise at levels below 110 dB SPL as their high-level stimuli. In four recent experiments, for example, high-level exposures consisted of an octave band noise (OBN) centered at 0.5 kHz at a level of 106 dB SPL for 48 h (McFadden et al., 1997), an OBN centered at 1.4 kHz at 105 dBA for 3 days (Skellett et al., 1998), an OBN centered at 2 kHz at 107 dBA for 48 h (White et al., 1998), and a 6.3 kHz tone at 100 dB SPL for 24 h (Canlon and Fransson, 1998). Long-duration stimuli such as these are presumed to induce cochlear damage by disrupting normal metabolic processes. In contrast, extremely brief stimuli (impacts and impulses) at levels above 120 dB damage the cochlea by a combination of metabolic and mechanical processes (Luz and Hodge, 1971; Hamernik et al., 1984). In real life, many individuals develop NIHL as a result of exposure to impact noises in industrial settings and impulse noises produced by gunfire and explosions, particularly in the military. Therefore, it is important to know if sound conditioning can provide protection from brief, high-level noises as well as from continuous tones and noise.

We are aware of only one published study of protection from impulse noise. Henselman et al. (1994) pre-exposed chinchillas to an OBN centered at 0.5 kHz at a level of 95 dB SPL for 6 h/day for 10 days. The animals were allowed to recover for 5 days, then they were exposed to impulse noise simulating M16 rifle fire at a level of 150 dB peak SPL. When assessed 30 days later, conditioned chinchillas were found to have significantly less PTS and smaller hair cell lesions than control animals exposed to the impulse noise alone.

The present experiment has two primary aims. The first aim is to determine if significant protection from impulse noise can be achieved with a shorter conditioning regimen than that used by Henselman et al. (1994). The second aim is to determine if there are differences between female and male chinchillas in their response to impulse noise, or in their ability to develop resistance to NIHL through sound conditioning. This is an important issue to address in light of previous studies showing gender differences in susceptibility to NIHL in humans (Gallo and Glorig, 1964; Berger, Royster and Thomas, 1978; Ward, 1966) and sex differences in PTS in chinchillas exposed to impulse noise (McFadden et al., 1999). In the latter study, female and male chinchillas were exposed to impulse noise at 150 dB peak SPL and PTS was assessed 30 days later. Female chinchillas developed approximately 10 dB more high-frequency hearing loss, but approximately 5 dB less low-frequency hearing loss than male chinchillas. On average, cochleas from females had 18% less inner hair cell (IHC) loss and 15% less outer hair cell loss (OHC) than males. The results suggested that there are sex/gender differences in the response of the cochlea to acoustic overstimulation that cannot be attributed to prior noise exposure history or other confounding factors.

I. METHODS

A. Subjects and Surgery

Subjects were 7 female and 7 male adult chinchillas obtained from a commercial breeder. Each subject was anesthetized with an intramuscular injection of ketamine hydrochloride (54 mg/kg) and acepromazine (0.64 mg/kg). A midline incision was made through the skin overlying the skull, and a small hole was made in the dorsal cranium overlying each inferior colliculus (IC). A tungsten electrode, approximately 2.5 cm long, was inserted through the hole and advanced through the IC to a depth that produced a clear, large-amplitude response to an 80 dB SPL click. The electrode was cemented to the skull with cyanoacrylic adhesive and dental cement. A short tungsten electrode, approximately 1.25 cm long, was implanted in the rostral cranium to serve as the common lead for evoked potential (IC-EVP) recording. Animals were allowed to recover at least 10 days before testing. All procedures were reviewed and approved by the University of Buffalo Animal Care and Use Committee, and conformed to federal guidelines for the humane treatment of laboratory animals.

B. Evoked potential test procedures

The awake chinchilla was placed in a restraining device (Snyder and Salvi, 1994) in a foam-lined sound attenuating booth. Stimuli were digitally generated tones (2 ms rise/fall, 10 ms duration, 20/s rate) converted to analog signals by a 16-bit D/A converter on a digital signal processing (DSP) board in a personal computer. Stimuli were routed through a computer-controlled attenuator and impedance matching transformer to a loudspeaker (Realistic 401197) in the test booth. The speaker was located on the side of the animal's test ear, approximately 9 cm from the animal's pinna. The non-test ear was plugged with a foam insert earplug. Stimuli were

presented in ascending order of frequency (in octave steps from 0.5 kHz to 16 kHz) and intensity (in 5 dB steps). Responses (electrical activity from the recording electrode in the IC contralateral to the test ear) were amplified (20,000X) and filtered (10-3000 Hz) by a Grass P511 bioamplifer and converted to digital signals by an A/D converter on a separate DSP board in the computer. Responses were computer averaged for 100 stimulus presentations. Threshold was defined as the mid-point between the level at which there was a clear deflection in the waveform and the next lower level at which there was none.

IC-EVPs were recorded (a) prior to noise exposure in order to establish pre-exposure baselines, (b) during the conditioning exposure in order to monitor temporary TS, (c) 5 days after conditioning to document TS recovery, (d) 15 min, 24 hr, and 5 days after impulse noise exposure in order to monitor temporary TS, and (e) after 20 days recovery from high-level exposure to determine PTS. Pre-exposure and PTS measures for each animal represent the average of three tests performed on separate days. Threshold shifts were calculated by subtracting mean pre-exposure IC-EVP thresholds from post-exposure thresholds.

C. Noises and noise exposures

Animals were exposed to 0.5 kHz OBN for 5 days (6 h/day) at a level of 95 dB SPL, followed 5 days later by impulse noise simulating M16 rifle fire (Danielson et al., 1991; Henselman et al., 1994; McFadden et al., 1999). The 0.5 kHz OBN was digitally generated, low-pass filtered (TDK HAF0030 active filter set at 20 kHz), manually attenuated (Hewlett Packard 350D), amplified (NAD 2200), and delivered to a compression driver (JBL 2446J) fitted to a bi-radial exponential horn (JBL 2360H). The driver/horn assembly was suspended from the ceiling of a sound booth. Animals were housed in separate cages (approximately 27 cm X 21 cm X 22

cm) placed beneath the loudspeaker, and provided free access to food and water during noise exposure. For acoustic calibration, sound pressure levels were measured with a calibrated Type I precision sound level meter (Larson-Davis 800B) and a 1/2" condenser microphone positioned at a height corresponding to the level of the ear canal of a standing chinchilla. SPL measurements were averaged across 5 positions within each cage (geometric center and each corner). Attenuator settings and cage positions were adjusted so that the average SPL in each cage was within 1 dB of the specified SPL. Animals were rotated to different cages each day to minimize any effects of slight differences in SPL between cages.

The impulse noise was generated digitally, converted to an analog signal by a 16-bit D/A converter on a DSP board, attenuated (Hewlett-Packard 350D), amplified (NAD 2200) and routed in parallel to two compression drivers (JBL 2446) in a sound booth. Each driver was fitted with a sound delivery tube (5 cm diameter X 20 cm) with its end angled at 45° to broaden its range of resonance (Danielson et al., 1991). The ends of the sound delivery tubes were separated by 10 cm. The animal in a restraining tube was placed between the drivers with the tubes directed at the animals' ears. Each animal was exposed to 50 pairs of impulses ("salvo" exposure), with 50 ms between the two impulses of each pair and a 1000 ms interval between the onset of each pair. The total exposure time was 50 s. The impulse noise was calibrated using a 1/8" microphone (Bruel & Kjaer 4138) and voltmeter to a level corresponding to 150 dB peak SPL.

D. Cochlear analyses

After the completion of testing, chinchillas were deeply anesthetized with sodium pentobarbital (Somlethal, 400 mg/kg i.p.) and decapitated. The cochleas were quickly removed

and perfused through the oval window with a succinate dehydrogenase (SDH) staining solution consisting of 2.5 ml of 0.2 M sodium succinate, 2.5 ml of 0.2 M phosphate buffer (pH 7.6), and 5 ml of 0.1% tetranitro blue tetrazolium. Cochleas were incubated in the SDH staining solution for 45 min at 37 °C, post-fixed with 10% formalin, and stored in fixative for at least 24 hours. Stained cochleas were dissected from the apex to the base, mounted in sections in glycerin on microscope slides, coverslipped, and examined using light microscopy (400X magnification). The numbers of missing OHCs and IHCs were determined for successive segments of the organ of Corti. Individual cochleograms were constructed to show the percentage of hair cells missing as a function of distance from the apex of the cochlea. Percent hair cells missing was referenced to our lab standards based on normal chinchillas. Percent distance from the apex was converted to frequency using the frequency-place map of Greenwood (1990).

E. Data analysis

One male and two female chinchillas lost one or both IC recording electrodes during the experiment. Data from these ears were excluded from analysis. The final sample consisted of data from 11 ears of females and 13 ears of males. Analyses of variance (ANOVAs, SPSS) were used to assess differences between means, with IC-EVP thresholds and threshold shifts as dependent variables. Independent variables were Sex (a between-subjects factor), Frequency and Time (within-subjects factors). Significant main effects and interactions involving Sex were analyzed further using independent Student *t*-tests. Changes as a function of time were assessed using paired *t*-tests. In order to determine if sound conditioning produced significant protection from impulse noise, data obtained from animals in this experiment were compared to data obtained from a control group of 9 female and 9 male chinchillas exposed to impulse noise alone under identical conditions, described in a previous publication (McFadden et al., 1999).

Comparisons between groups were made using separate two-way Group X Sex ANOVAs for mean low-frequency PTS (average of PTS values at 0.5, 1 and 2 kHz) and high-frequency PTS (average of PTS values at 4, 8 and 16 kHz).

II. RESULTS

A. Pre-exposure thresholds

Pre-exposure thresholds are shown in Figure 1. A two-way (Sex X Frequency) mixed ANOVA showed a significant Sex X Frequency interaction, $F(5,110)=2.62$, $p = 0.028$, due to the reversal of threshold differences between low and high frequencies. Student t-tests at each frequency showed that the difference between females and males at 16 kHz was statistically significant, $t(22)=2.68$, $p=0.014$. Pre-exposure thresholds were similar to those of control animals in our previous study (McFadden et al., 1999).

B. Threshold shifts from conditioning noise

Threshold shifts due to sound conditioning are shown in Figure 2. After the first day of sound conditioning, thresholds were elevated by approximately 30-40 dB SPL at low frequencies, and by 0-25 dB at high frequencies. Thresholds were significantly elevated at all frequencies for females and at all frequencies except 16 kHz for males (paired t-tests; all p values < 0.04). Two-way (Sex X Time) mixed ANOVAs for TS at each frequency yielded significant main effects of Sex at 1, 2, 4 and 16 kHz ($F(1,22) = 5.17$, 6.64, 7.02, and 8.35, respectively; all p values < 0.04) and main effects of Time at all frequencies except 16 kHz (all p values < 0.02). As shown in Figure 2, the main effect of Sex arose because females consistently showed more TS than males during and after the conditioning exposure.

Paired *t*-tests were used to follow-up on the significant main effects of Time. These analyses indicated that TS decreased significantly at 0.5 kHz between Day 1 and Day 5 of conditioning for both males, $t(12)=2.84$, $p = 0.015$, and females, $t(10)=2.97$, $p=0.014$. The decreases in TS that occurred between the last day of conditioning and the end of the 5-day recovery period were significant at all frequencies except 16 kHz (all p values < 0.04). After 5 days of recovery from conditioning, thresholds were within approximately 5 dB of pre-exposure values at all frequencies (Fig. 2, bottom panel).

C. Threshold shifts after impulse noise

Mean TS values at 15 min, 24 h and 5 days after impulse noise exposure are shown in Figure 3. Two-way (Sex X Time) mixed ANOVAs for TS at each frequency showed significant Sex X Time interactions at 2 and 4 kHz ($F(3,66) = 5.36$ and 3.14, respectively, p values < 0.04), and significant main effects of Time at all frequencies (all p values < 0.001). As shown in Figure 3, the interactions occurred because females showed more recovery at 2 and 4 kHz over time than males.

Mean PTS is shown in Figure 4. Paired *t*-tests indicated that impulse noise produced significant PTS at all frequencies for both males and females (all p values ≤ 0.01). A two-way (Sex X Frequency) ANOVA showed a significant Sex X Frequency interaction, $F(5,110)=4.20$, $p = 0.002$. The interaction occurred because males developed more PTS than females at low frequencies, but less PTS than females at 16 kHz.

To summarize the IC-EVP test results, females had a significantly lower mean threshold at 16 kHz than males prior to exposure (Fig. 1). During conditioning, females consistently showed greater TS than males, but thresholds of both sexes were essentially normal within 5 days after

conditioning (Fig. 2). Subsequent exposure to M16 rifle fire produced more TS at low-frequencies for males, and more TS at high frequencies for females (Fig. 3). When PTS was assessed 20 days after exposure to M16 rifle fire, females showed approximately 5-10 less PTS than males at 0.5, 1 and 2 kHz, but approximately 15 dB more PTS than males at 16 kHz.

D. Noise-induced hair cell losses

Mean cochleograms are shown in Figure 5. Hair cell lesions were slightly smaller in cochleas from females. Average OHC losses in the apical half of the cochlea were approximately 30% for females and 40% for males. Average OHC losses in the basal half were approximately 50% for females and 60% for males. The most striking difference was seen in the 2 kHz region of the cochlea, where males showed approximately 30% more OHC loss than females. Mean IHC losses were not remarkably different between females and males. Averaged across the entire cochlea, females had 11% IHC loss and 38% OHC loss, whereas males had 14% IHC loss and 47% OHC loss.

E. Protection from M16 rifle fire

One purpose of exposing animals to the 5-day conditioning regimen was to provide protection from subsequent exposure to M16 rifle fire. A perspective on the success of this approach is provided by Figure 6, which shows differences in PTS between conditioned animals in the present study and control animals exposed only to the impulse noise (McFadden et al., 1999). Sound conditioning provided up to 18 dB protection for females and up to 10 dB protection for males at individual frequencies. Collapsed across sex, the protective effect was 5 to 12 dB across frequencies, with greater protection at low frequencies than at high frequencies.

Figure 7 compares average low-frequency PTS and high-frequency PTS for conditioned and control animals. The pattern of PTS was similar for conditioned animals and controls. For both groups, females showed less low-frequency PTS but more high-frequency PTS than males. Conditioned females and males both showed less PTS than their same-sex controls. Two-way (Group X Sex) ANOVAs showed significant main effects of Group, $F(1,51)=6.70$, $p=0.012$, and Sex, $F(1,51)=4.70$, $p=0.035$, for low-frequency PTS. Thus, conditioned animals showed significantly less low-frequency PTS than controls, and females showed significantly less low-frequency PTS than males. Despite the consistent trends for high-frequency PTS, neither differences between males and females nor differences between conditioned animals and controls were statistically significant.

Hair cell lesions were smaller in the conditioned animals compared to controls. Conditioned males had approximately 20% less IHC loss and 24% less OHC loss than control males. Conditioned females had approximately 5% less IHC loss and 18% less OHC than control females. Collapsed across sex, sound conditioning resulted in approximately 12% less IHC loss and 21% less OHC loss compared to controls.

III. DISCUSSION

A. Protective effects of sound conditioning

The results show that sound conditioning provides protection from PTS and hair cell loss caused by impulse noise exposure. Chinchillas conditioned with a 0.5 kHz OBN at 95 dB SPL for 6 h/day for 5 days developed approximately 5-12 dB less PTS across frequencies, and 13-21% less hair cell loss than chinchillas exposed to the impulse noise alone. As shown in Figure 7, protection was apparent at high frequencies (approximately 6 dB overall) as well as low

frequencies (approximately 10 dB overall). However, only the protection at low frequencies reached statistical significance.

The present results confirm and extend the results of an earlier study by Henselman et al. (1994) by showing that a 5-day conditioning regimen can protect the ear from impulse noise. A comparison between the two studies also shows a "dose effect" related to the duration of the conditioning exposure. In the Henselman experiment, a 10-day conditioning regimen produced approximately 7-23 dB of protection across frequencies, with the greatest protection (20-23 dB) at 2 and 4 kHz. Averaged across frequencies, the protective effect of a 10-day conditioning regimen was approximately 16 dB SPL. The 5-day conditioning regimen used in the present study also provided significant protection from PTS. However, the magnitude of protection was approximately 6-10 dB less than that provided by the longer conditioning exposure. Differences related to the duration of the conditioning exposure are also apparent in hair cell losses. The pattern of hair cell loss we observed in the present study was similar to the pattern seen by Henselman et al. (1994). However, the magnitude of hair cell protection was considerably greater for the 10-day conditioning regimen. Animals conditioned for 5 days (present study) had approximately 40% OHC loss across the entire cochlea, whereas animals conditioned for 10 days (Henselman et al., 1994) had less than 20% OHC loss. The "dose effect" of conditioning is interesting because it indicates that the magnitude of protection can be increased by extending the "training" period. The mechanisms by which sound conditioning increases resistance to NIHL are not known, but they are clearly not "all or none" phenomena. Previous studies have indicated dose effects of sound conditioning related to exposure duration (Canlon and Fransson, 1998; Subramaniam et al., 1993) and rest period between conditioning and high level exposure (McFadden et al., 1997; Subramaniam et al., 1992).

It is interesting that sound conditioning can protect the cochlea from impulse noise, which can damage the cochlea by causing direct mechanical failure as well as through metabolic disruption. Whether sound conditioning actually increases resistance to mechanical damage, or whether it only attenuates damage brought about by metabolic changes is not clear. However, there is some evidence that the structural components of the ear may be altered by sound conditioning in a way that could afford protection from mechanical damage (Hu and Henderson, 1997; Pack et al., 1999).

B. Differences between female and male chinchillas

The results show significant differences between female and male chinchillas in their response to noise. During sound conditioning, females consistently showed greater TS than males. After impulse noise exposure, females developed approximately 10 dB less PTS at low frequencies, but approximately 5 dB more PTS at high frequencies than males. The difference in low-frequency PTS between females and males was statistically significant. The pattern of PTS in sound conditioned animals was very similar to that reported previously for animals exposed to impulse noise alone (McFadden et al., 1999). In both cases, females had less TS at low frequencies and greater TS at high frequencies than males. Sound conditioning provided significant protection for both females and males, with little difference in the magnitude of protection.

The reasons for the sex differences cannot be determined from this study. However, since the differences were observed in chinchillas, extraneous factors such as differences in noise exposure history, recreational activities, and dietary factors can be ruled out. One possibility is that sex/gender differences in susceptibility to NIHL arise from differences in the acoustical

properties of the outer and middle ears. A second possibility is that sex/gender differences arise from basic physiological differences between female and male cochleas. Interestingly, Mills et al. (1999) recently reported that male rats are more susceptible to kanamycin ototoxicity than female rats, a difference that clearly cannot be attributed to differences in acoustical properties of the ear. McFadden et al. (1998) observed changes in otoacoustic emissions in a human male treated with estrogen, indicating that sex hormones can influence outer hair cell function. Because the outer hair cell system is a likely candidate as a site for conditioning-induced changes (Canlon, 1996; Canlon and Fransson, 1995; Hu and Henderson, 1997), observations such as this may be important for understanding the sex differences we have observed in chinchillas. Future studies using the chinchilla may help determine the relative importance of anatomical and physiological factors in sex/gender differences in sound conditioning and susceptibility to NIHL.

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Figure Legends

Figure 1. Mean thresholds of female (solid circles) and male chinchillas (open triangles) before noise exposure. Bars in this and subsequent figures represent standard errors of the means (SEM).

Figure 2. Mean threshold shifts (± SEM) during and after sound conditioning. Top panel: threshold shifts after the first day (6 h) of sound conditioning. Middle panel: threshold shifts after the last day of conditioning. Bottom panel: threshold shifts 5 days after the last conditioning exposure and before exposure to impulse noise.

Figure 3. Mean threshold shifts (± SEM) at three times after impulse noise exposure. Top panel: threshold shifts 15 min after impulse noise exposure. Middle panel: threshold shifts one day after impulse noise exposure. Bottom panel: threshold shifts 5 days after impulse noise exposure.

Figure 4. Permanent threshold shifts (PTS) measured 20 days after impulse noise exposure for females (solid circles) and males (open triangles). Bars show SEMs.

Figure 5. Cochleograms showing hair cell loss after impulse noise exposure. Left panel: outer hair cell (OHC) losses for males (solid lines) and females (dashed lines). Right panel: inner hair cell (IHC) losses for males (solid lines) and females (dashed lines).

Figure 6. Protection from permanent noise-induced hearing loss afforded by sound conditioning in females (solid circles) and males (open triangles). Protection is the difference in permanent threshold shift (PTS) between control animals exposed to the impulse noise alone and animals conditioned with 0.5 kHz octave band noise at 95 dB SPL for 5 days (6 h/day).

Figure 7. Mean permanent threshold shifts (PTS) at low frequencies (average of 0.5, 1 and 2 kHz) and high frequencies (average of 4, 8 and 16 kHz) for conditioned females and males and their same-sex controls. Bars show SEMs.

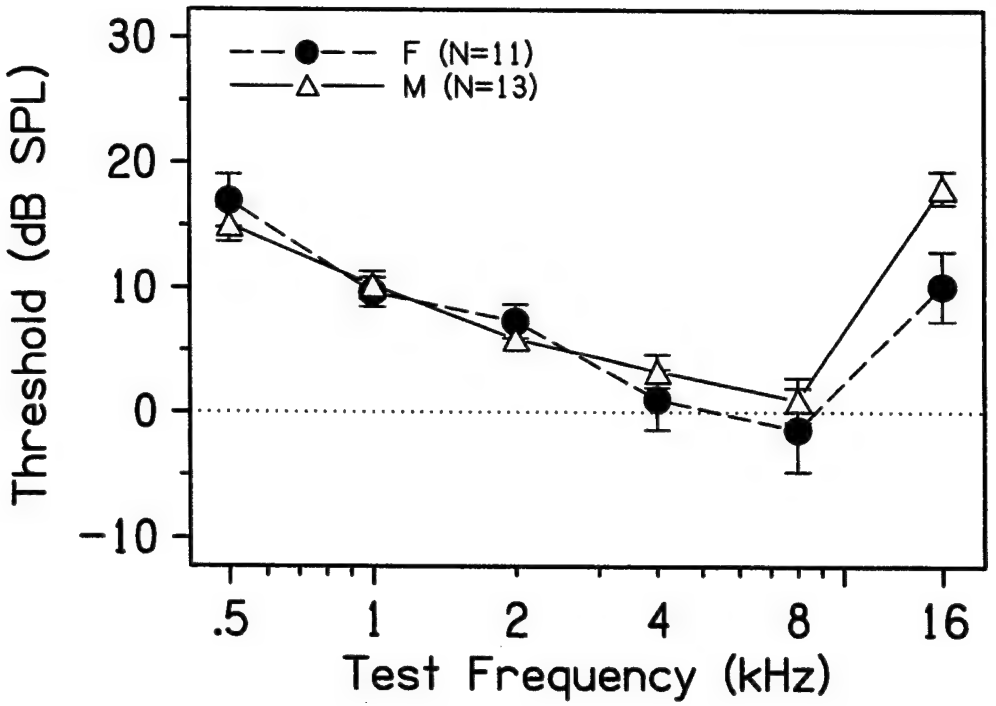
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Figure 1

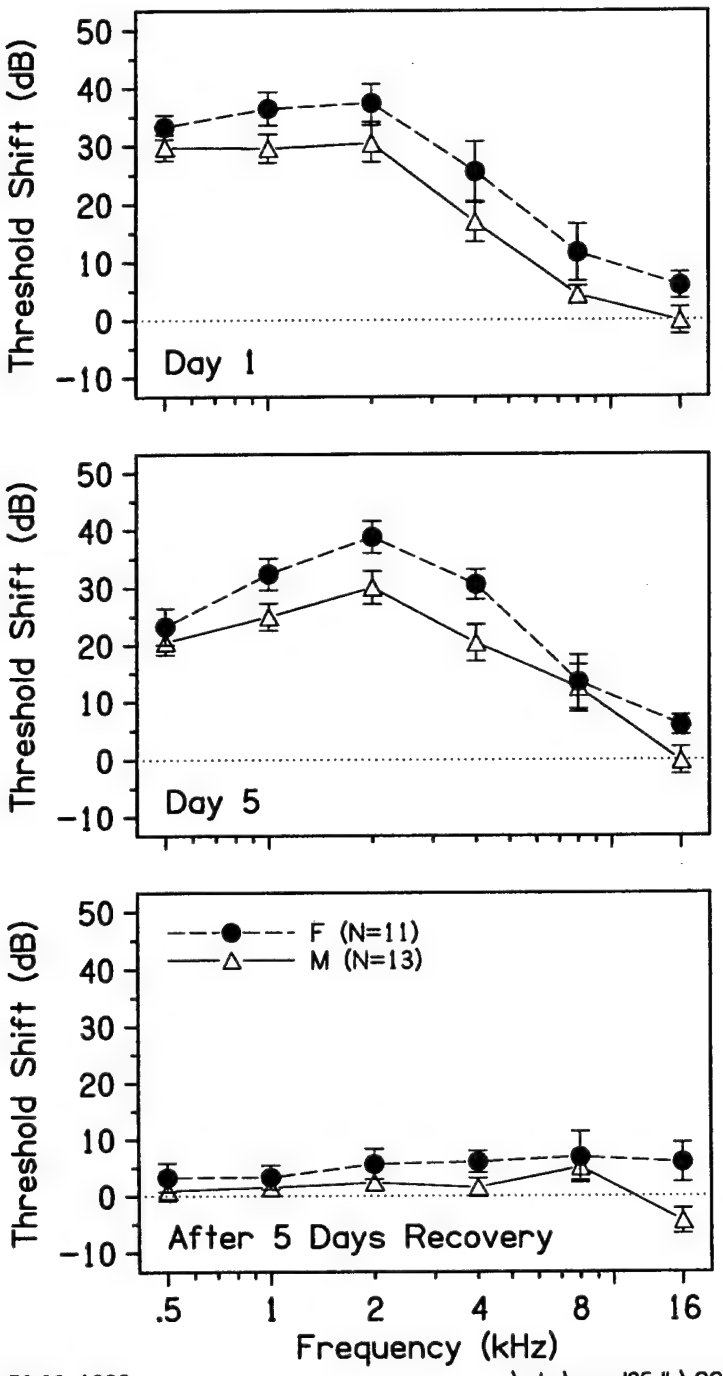

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Figure 2

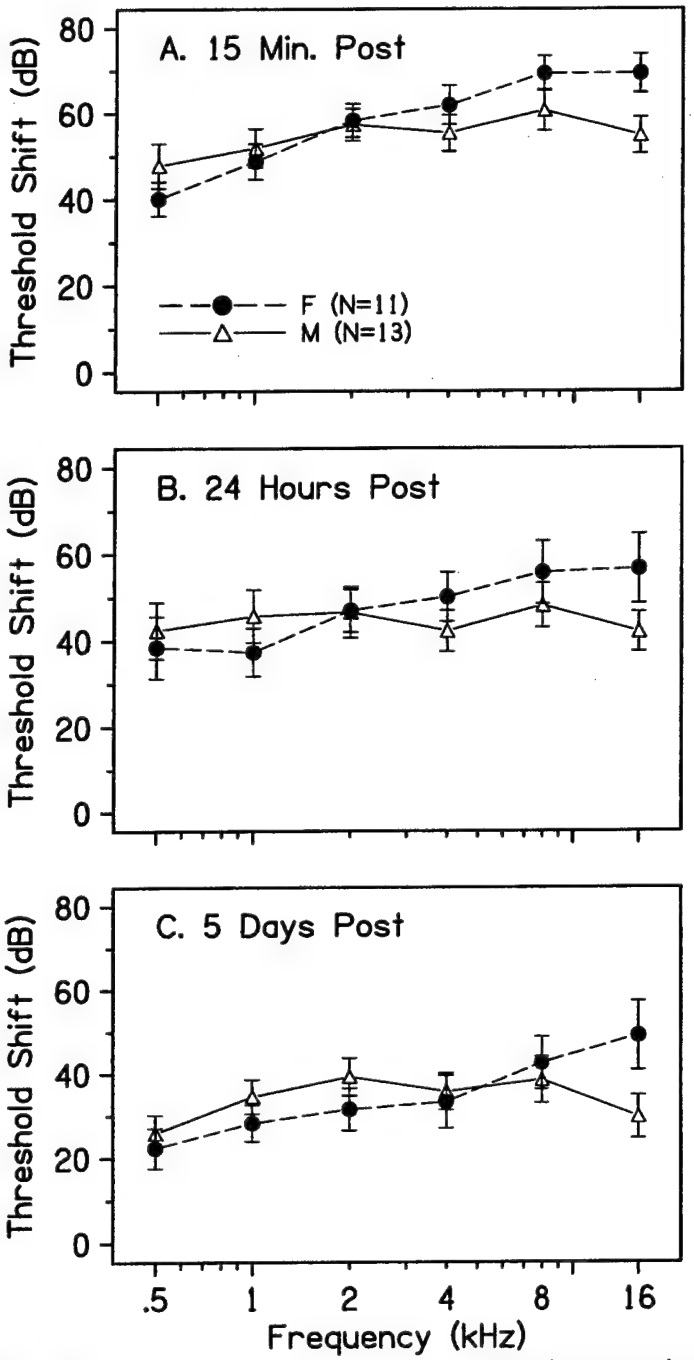

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Figure 3

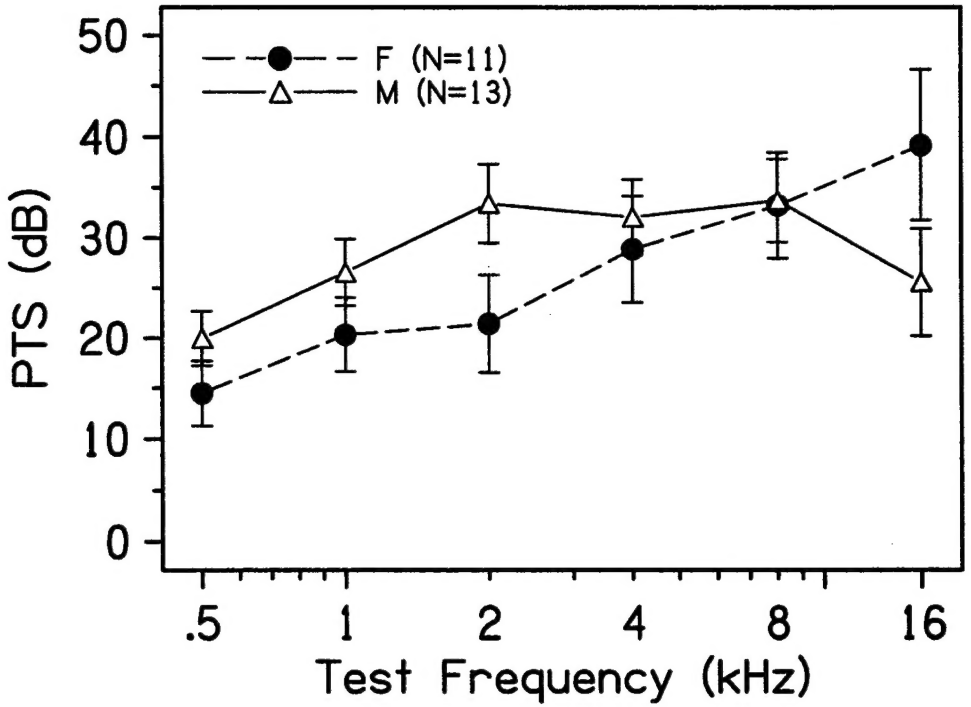
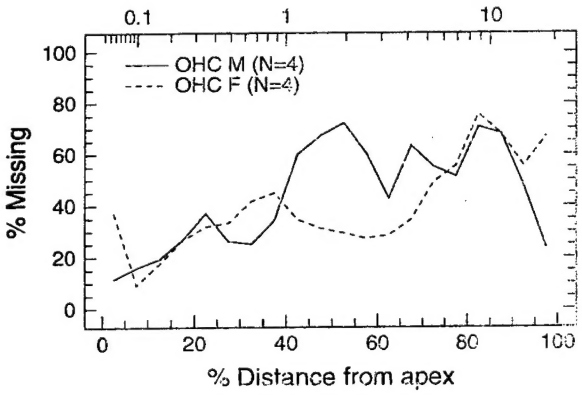


Figure 4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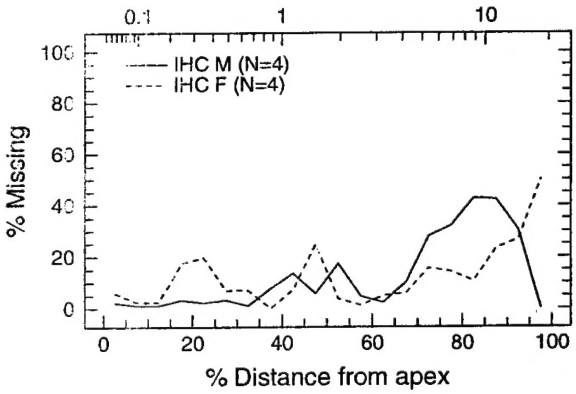
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% Distance from apex

Figure 5

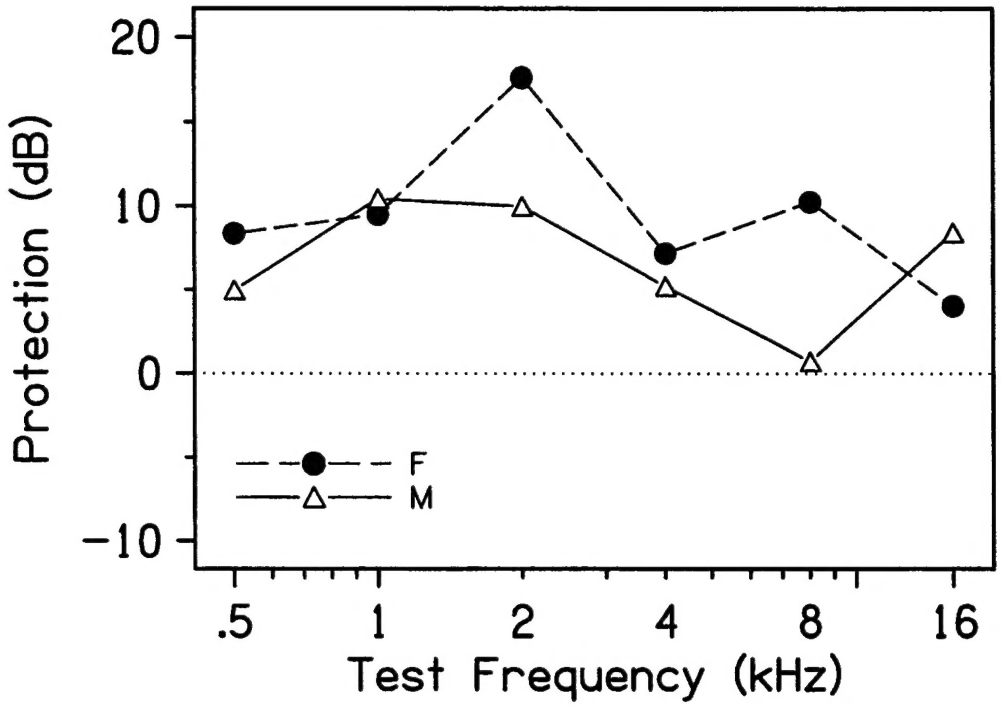

Figure 6

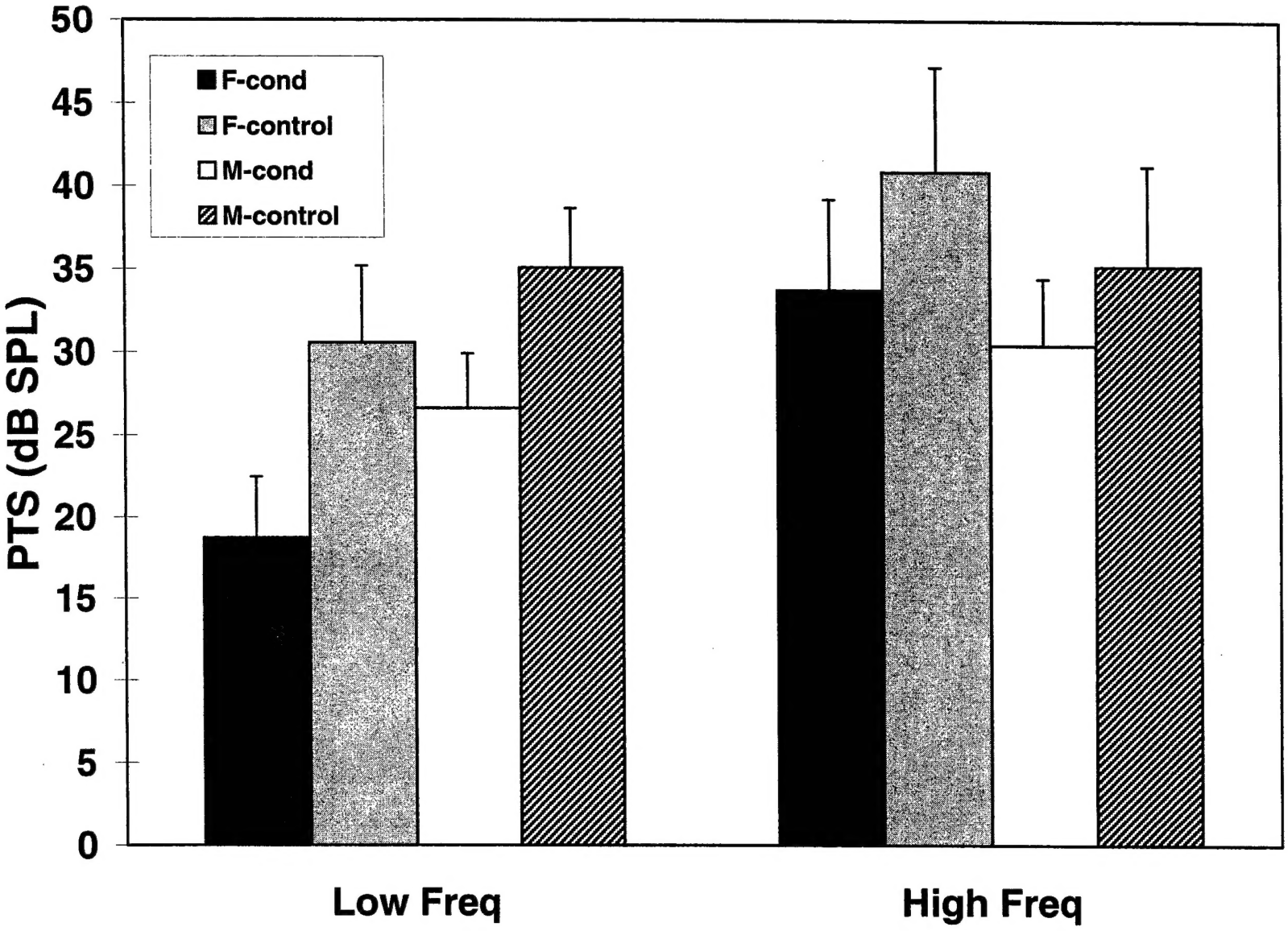
50
45
40
35
30
25
20
15
10
5
0
PTS (dB SPL)
F-cond
F-control
M-cond
M-control
Low Freq
High Freq

Figure 7